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# Evaluation of Aquatic Herbicides for Selective Control of Variable Milfoil (*Myriophyllum heterophyllum* Michx)

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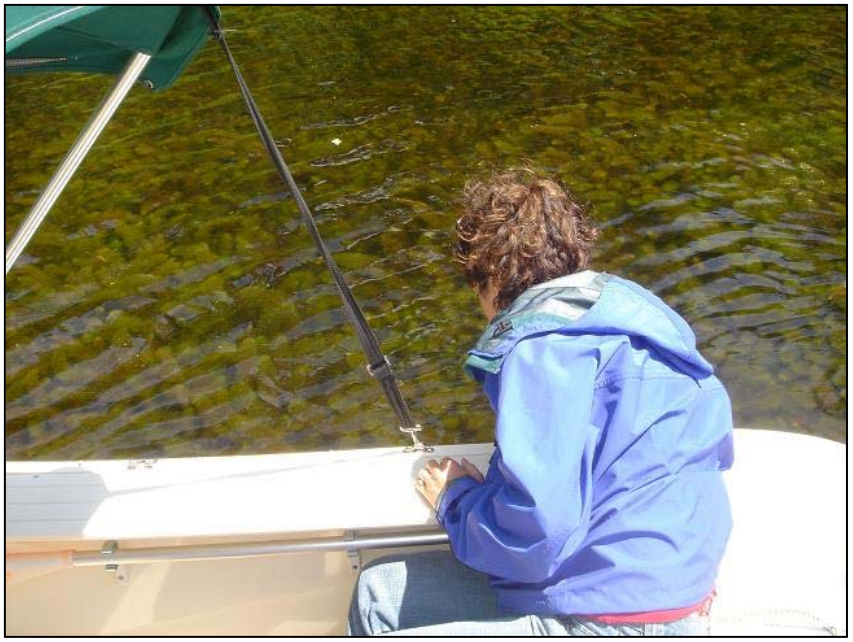

**Table of Contents**

**Summary of Research Findings:**

The native submersed plant variable milfoil (*Myriophyllum heterophyllum* Michx) can be found in numerous water bodies in the United States, yet it is considered an invasive species in the northeast and the state of New Hampshire. In order to provide improved guidance for control of variable milfoil, we conducted a series of laboratory and mesocosm studies to evaluate the efficacy of various registered and experimental use aquatic herbicides. At the time of the initial proposal we identified seven active ingredients for evaluation; however, due to the recent expansion of Experimental Use Permits for aquatic use, we screened a total of 12 active ingredients. Variable milfoil was collected from numerous sites in New Hampshire, and comparative phytotoxicity studies with 2,4-D, diquat, and triclopyr conducted at the University of Florida Center for Aquatic and Invasive Plants (CAIP) showed no significant trend in intra or inter-site difference in the response to herbicides between different populations. This result allowed us to pool our variable milfoil cultures into a single group and focus future research on the response of this species to selected herbicides and formulations. A series of concentration and exposure time trials were conducted comparing the efficacy of the contact herbicides carfentrazone-ethyl, chelated copper, diquat, endothall, and flumioxazin. Results indicated that the protoporphyrinogen oxidase enzyme inhibitors carfentrazone and flumioxazin provided superior efficacy when compared to the maximum label rate of diquat and various diquat and copper combinations under a variety of exposure scenarios. Endothall and chelated copper were found to be largely ineffective at the rates and exposures tested. The growth regulating systemic herbicides, 2,4-D butoxyethylester (BEE), 2,4-D amine, triclopyr, and quinclorac all provided good control of variable milfoil across a broad range of concentrations and exposures tested. However under most scenarios evaluated, the 2,4-D BEE provided superior control compared to the other products. Our study protocols suggest that the combination of the ester formulation and low alkalinity waters contributes to the enhanced efficacy of 2,4-D BEE. Direct comparison of equivalent use rates of 2,4-D BEE and a granular amine formulation of triclopyr provided further evidence that the ester component is more important to enhanced efficacy versus the use of a liquid or granular formulation. We

also conducted testing of several slow acting enzyme-inhibiting herbicides including the phytoene desaturase (PDS) inhibitor fluridone, and the acetolactate synthase (ALS) inhibitors bispyribac, imazamox, and penoxsulam. While these compounds allow for very low use rates, they require exposure periods of several weeks in order to control the target plants. These products would likely be used at low rates for whole-lake treatments similar to current Eurasian watermilfoil treatment strategies. In our studies, fluridone and penoxsulam were quite active on variable milfoil at concentrations as low as 5 $\mu g\ L^{-1}$, whereas bispyribac and imazamox did not show herbicidal activity at the concentrations evaluated. Upon completing the evaluations of all of the herbicides, we further evaluated the efficacy of 2,4-D BEE, 2,4-D amine, triclopyr amine, and carfentrazone at water temperatures ranging from 13 to 25 C. Within the range evaluated, water temperature did not have a significant impact on treatment efficacy of any of these compounds. Results suggest that actively metabolizing plants were equally susceptible to the herbicide treatments.

During the course of our work, we found that variable milfoil could survive as a small emergent form for periods of several months upon removal from water. Subsequent evaluations showed that these plants would quickly revert to a submersed morphology and begin growing to the water surface upon resubmersion. Treatment of the emergent variable milfoil with a 1.5 to 2% solution of 2,4-D or triclopyr amine and a nonionic surfactant resulted in good control of the plants when they were submerged again.

In summary, the overall results of all of our studies largely validate the use of 2,4-D BEE as a primary tool for variable milfoil control. The dicot selective properties of the growth regulating systemics and the ability to control the entire plant due to translocation of herbicide into the rootcrown are key advantages of these products. Our studies also demonstrated that new enzyme-specific contact herbicides such as carfentrazone and flumioxazin, have short exposure requirements and are likely to provide improved control over diquat in many situations. Efficacy of carfentrazone was also validated in the field on variable milfoil populations located in North Carolina. The lack of efficacy of endothall and chelated copper in our trials would discourage field use or evaluations of

these products in New Hampshire. Our work suggests that the variable milfoil growing in New Hampshire lakes is not particularly tolerant to aquatic herbicides and it can be controlled at label use rates by a number of the registered aquatic herbicides. Treatment strategies will differ depending on the size of the water body, size of the plant infestation, uses of the water, and the desired length of control. The tools available allow for both small-scale treatments where a new infestation is found, as well as large-scale treatments for plants that have been established for many years.

**General Introduction:**

Variable milfoil (*Myriophyllum heterophyllum* Michx) is a native perennial aquatic plant ranging from southwestern Quebec and Ontario to North Dakota and southward to New Mexico and Florida (Godfrey and Wooten 1981). This submersed dicotyledon is classified as a species of concern in Kentucky and is endangered in Ohio and Pennsylvania (USDA 2007). In the northeastern U.S. however, variable milfoil is not native and is considered an invasive and weedy species. It represents a particular threat to the numerous low alkalinity and relatively acidic water bodies in this region. Variable milfoil is listed as invasive in states such as Connecticut and Maine, prohibited in Massachusetts, and is a class A noxious weed in Vermont (USDA 2007). As an invasive species, it causes many of the same problems as Eurasian watermilfoil (*Myriophyllum spicatum* L.), including shading out other native submersed vegetation and interfering with recreational activities and water supplies (NH-DES 2002; Halstead et al. 2003). It has also been estimated that variable milfoil could reduce lake-front property values by as much as 20 to 40 percent in New Hampshire (Halstead et al. 2003).

Using the spread and establishment of the closely related Eurasian watermilfoil as a model for invasion of the higher alkalinity water bodies in the northeast, the long-term future of New Hampshire's native aquatic plant community depends upon the development and implementation of effective and environmentally compatible strategies for managing existing infestations and eliminating pioneer infestations of variable milfoil. The significant reduction or elimination of established populations is important, as these stands are likely serving as the major source of spread for this plant. Current operational options for controlling invasive plants like variable milfoil may include

mechanical and hand harvesting methods, but the environmentally sound use of herbicides probably offers the most expedient and cost-effective means to reduce or remove large infestations and halt the expansion of pioneer infestations (Madsen 2000).

A range of particularly troublesome invasive submersed plants, such as Eurasian watermilfoil, curlyleaf pondweed (*Potamogeton crispus* L.), and hydrilla (*Hydrilla verticillata* L.f. Royle), are frequently managed using aquatic herbicides. Herbicide use is particularly important when a rapid and significant reduction of established populations, and/or eradication of pioneer infestations, is required to prevent an invasive species from spreading to other water bodies. Larger scale herbicide treatment strategies must consider both control of the target species and response and recovery of the native plant community. These treatments take into account inherent herbicide selectivity, use rates, timing, and the composition of the native plant community to provide the maximum impact on the target species while minimizing injury to the native plant communities (Getsinger et al. 1997; Parsons et al. 2001; Getsinger et al. 2002b; Madsen et al. 2002; Skogerboe and Getsinger 2002; Poovey et al. 2004; Getsinger et al. 2007).

There are a number of chemical products (contact and systemic) currently registered for controlling milfoils, and several more active ingredients are under review for Section 3 aquatic labels by the US Environmental Protection Agency (EPA). Reports of herbicide efficacy for control of variable milfoil are very limited compared to the widespread herbicide literature available for Eurasian watermilfoil. This disparity is likely due to the exotic status of Eurasian watermilfoil (introduced from Eurasia) and the longer history and larger number of northern tier lakes infested with this invasive plant. In its native range, variable milfoil is not considered particularly weedy, although it can grow to nuisance levels in some aquatic sites. It is interesting to note that Eurasian watermilfoil has not spread to New Hampshire waters despite long-term and widespread presence in several surrounding states. Based on the distribution of variable milfoil in its native range, it is thought that the water quality conditions in many Northeastern lakes (low alkalinity and acidic waters) would favor a species such as variable milfoil over Eurasian watermilfoil (Hoyer et al. 1996). Our initial attempts to culture variable milfoil and

Eurasian watermilfoil in the same tanks at the UF CAIP inevitably lead to one species dominating the other. The only significant difference in culture conditions was the alkalinity and pH that was maintained. Variable milfoil growth was strongly favored in the low alkalinity/low pH environment, whereas Eurasian watermilfoil was dominant in the higher alkalinity/high pH environments.

Uptake of herbicides by submersed plants primarily occurs via actively growing shoot tissue. Therefore, to control submersed plants the surrounding water column is treated with a target concentration of herbicide thus establishing a concentration and exposure time (CET) relationship. It has been proven that effective and selective control of submersed plants, including plants such as Eurasian watermilfoil, is directly related to CET relationships which are comprised of three critical factors: a) aqueous concentration of the herbicide surrounding the plant; b) exposure period or contact time of the herbicide with respect to the treated plant; and c) specific mode of action of a herbicide (Green and Westerdahl 1990; Netherland and Getsinger 1992; Getsinger 1998). Field experience suggests that variable milfoil can be controlled using herbicides, but documentation of herbicide CET relationships and comparative efficacy for various herbicides is lacking.

The literature that is available suggests that products such as 2,4-D BEE and triclopyr can provide control of variable milfoil (Bugbee et al. 2003**;** Getsinger et al. 2003). Initial CET relationships were developed for variable milfoil and triclopyr, and this approach should be further refined to include other systemic and contact herbicides as well as the slow acting enzyme inhibitors. Development of comparative efficacy data for registered and EUP compounds would provide resource managers with information that allows them to choose the best product for various site and situation specific treatment scenarios.

There are ten compounds currently registered for aquatic use by the US EPA that we screened for activity against variable milfoil. General chemical characteristics, terrestrial use patterns, information on the mode of action, and basic environmental toxicology information can be found in the Weed Science Society Herbicide Handbook (WSSA 2002). Compounds evaluated included 2,4-D (the granular butoxyethylester (BEE) and

liquid amine), carfentrazone, chelated copper, diquat, endothall, fluridone, penoxsulam, and liquid and granular amine formulations of triclopyr**.** Four other herbicides have recently received US EPA Experimental Use Permits (EUP) for evaluation in aquatic sites. These products include the acetolactate synthase inhibiting herbicides bispyribac and imazamox, the protoporphyrinogen oxidase (protox) inhibitor flumioxazin, and the auxin mimic quinclorac. A list of the herbicides, the date registered for aquatics, and the chemical name of the products is provided in Appendix 1**.** While the original proposal called for evaluation of only seven herbicides, all fourteen of the above-mentioned products (12 active ingredients) were evaluated for their activity against variable milfoil. The products represent three categories of use patterns: 1) auxin-mimic or growth regulating systemic herbicides that are inherently selective for many dicotyledons, with a moderate exposure requirement (12 to >48 hr) and use in both large-scale and spot-treatments: 2,4-D, triclopyr, and quinclorac; 2) relatively broad-spectrum contact herbicides that have short contact requirements (4 to >24 hrs): carfentrazone, chelated copper, diquat, endothall, and flumioxazin, and; 3) enzyme-specific systemic herbicides that allow for low appli-cation rates and extended contact time requirements (>60 days): fluridone, bispyribac, imazamox, and pensoxsulam. These enzyme-specific systemics are typically used for whole-lake treatments or in areas with limited water exchange (e.g. coves, marinas).

There were numerous studies conducted as part of this project, and some of this work has been written for submission to Journals. We have included papers submitted to Journals in the Appendices and we refer to these articles and study results in the appropriate portions of the text. Moreover, many trials were often more exploratory in nature, and we did not attempt to include the results of every study we conducted.

**Initial Plant Collections and Culturing:**

Our initial objective was to determine the optimal methods for culturing variable milfoil for use in laboratory and mesocosm studies. Variable milfoil shoot tissue was collected by NHDES personnel from several sites within the State of New Hampshire (Table 1) and sent to the UF CAIP in Gainesville, Florida during late Fall of 2004.

Table 1. Variable milfoil collection sites in New Hampshire.

| Site | Size (Ha) | County | Sites Sampled |
|---|---|---|---|
| Horseshoe Pond | 15.1 | Hillsborough | 3 |
| Lees Pond | 72.5 | Carroll | 3 |
| Turkey Pond | 65.8 | Merrimack | 3 |
| Lake Winnipesaukee (Wolfe Bay) | 18,043 | Belknap | 1 |
| Lake Massabesic | 1173.6 | Rockingham | 1 |

Healthy apical tips from these samples were excised and a total of four 15 cm sections were planted into 4 Liter pots that contained either an organic sediment (41% organic, 44% silt, 13% clay) collected from Bivens Arm Lake, FL, Vitahume potting soil (80% sand, 11% silt, and 8% organic), a mixture of 50/50 Vitahume potting soil and builders sand, or a 50/50 mixture of the potting soil and organic sediment from Bivens Arm. All of the sediments tested have been used in previous efforts at the CAIP to culture a wide variety of invasive and native submersed plants. All sediment mixtures were amended with Osmocote (15:9:12) at a rate of 2 g/Kg of dry sediment. Plants from different lakes were grown in separate 900 L concrete mesocosm tanks (Figure **1**), and each sediment type was labeled. Culture water was amended via addition of HCl to achieve an initial pH of 6.5 and alkalinity (A.N.C.) between 5 and 20. HCl was added periodically to keep pH and alkalinity within the desired range.

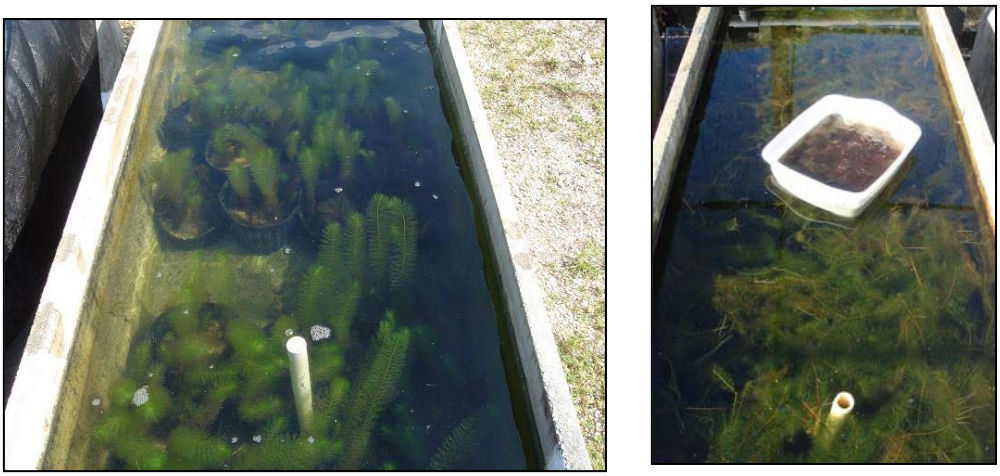

Figure 1. Culture tanks in Gainesville, FL used to grow Variable milfoil collected from several NH lakes.

While we evaluated the use of chillers to cool the water used for our culture tanks during the summer of 2005, we did not find any particular growth or culture health benefits occurred as a result of recirculating chilled water to keep water temperatures down. The chilling system resulted in maintaining water temperatures between 4 and 6 C cooler than water in ambient tanks. An overhead shade canopy (40% shade) kept the culture tanks from absorbing direct sunlight during the summer and this resulted in summer (June 1 through September 30) water temperatures in the non-chilled tanks being maintained between 21 and 28 C. Following the testing in the summer of 2005, all cultures were subsequently maintained under ambient temperatures. Culture plants grew very well and we still maintain several tanks that contain the original plants and pots established in late 2004. In contrast to Eurasian watermilfoil cultures that tend to grow rapidly and then crash within a few months, the variable milfoil cultures tend to remain quite stable in terms of biomass and health.

Variable milfoil collected from all 11 sites in New Hampshire grew very well under the culture conditions, and plants rapidly established on all of the sediments. Shoot meristems of 15 cm, readily took root in the sediment and shoots grew to the water surface (65 cm) and formed canopies within 1 month. We did note that once the plant formed a canopy, growth rates slowed considerably. Initial harvests suggested that variable milfoil grew the fastest in the 50/50 potting soil and muck mixture; however, the plants growing in the fertilized potting soil alone showed the best long-term culture properties. For our cultures, it was important that the plants withstand numerous clippings of apical meristems. All subsequent cultures and studies conducted at the UF CAIP utilized the potting soil and fertilizer rates described above.

We noted that plants in a few culture tanks were prone to developing dense epiphytic algal growth over time (this is commonly noted with plants in the field). Collecting the apical meristems of these plants, thoroughly rinsing them to remove as much of the attached algae as possible, and reestablishing these plants was the best method for maintaining clean culture plants. All of our study protocols called for the use of clean plant tissue for herbicide efficacy studies. It is interesting to note that two of our variable

milfoil culture tanks have sustained rather dense *Lyngbya* sp. populations over a 2-year period. While the *Lyngbya* never becomes dominant, it often becomes attached to the plants. For our efficacy trials, we avoided the use of plants that had significant amounts of *Lyngbya* attached.

For studies conducted at the US Army Engineer Lewisville Aquatic Ecosystem Research Facility (LAERF), variable milfoil was shipped from the CAIP prior to initiation of the studies. LAERF greenhouse facility chillers (Pacific Coast Imports) were used to regulate water temperature of the treatment tanks, and carbon dioxide was bubbled into the tanks to regulate pH of the water. Conditions maintained for the specific studies are described in the individual Materials and Methods sections.

**Initial Herbicide Testing – Evaluation of Intra-site and Inter-site Variation:**

*Methods:*

We chose the liquid herbicides 2,4-D amine, triclopyr amine, and diquat to conduct initial trials for evaluating the potential for intra-site or inter-site variation in response to herbicide treatment. On September 12, 2005 an individual 10 cm shoot of variable milfoil was planted in a 150 ml culture tube filled with fertilized potting soil (described above). Culture tubes containing variable milfoil were then placed in racks and were grown outdoors in 900 L concrete mesocosm tanks for a 3-week pre-treatment growth period (Figure **2**). Variable milfoil from each collection site within the 5 sample lakes was removed from the culture tank, placed in 95 L treatment tanks and exposed to either 2,4-D or triclopyr concentrations of 1.0 and 2.0 mg/L for 2, 6, 12, and 24 hr. Variable milfoil was exposed to diquat concentrations of 0.15, and 0.30 mg/L for 1.5, 3, 6, and 18 hr. A label stake was placed in each tube to denote the plant source, collection site, herbicide and rate used, and the exposure period. During the course of the herbicide exposures, water temperatures were maintained between 21 and 23 C, and pH was maintained between 6.2 and 6.7. Following the designated exposure period, plants in the

Figure 2. Variable milfoil growing in culture tubes.

tubes were thoroughly rinsed in untreated water and then placed back in racks in the 900 L culture tanks. Water was added to the 900 L tanks to achieve two complete exchanges of volume every 24 hr for 6 d post-treatment. This was done to prevent low-level residual herbicide leaching from the plants and building to levels that could confound study results. The plants were given a 21-d post-treatment recovery period. High and low water temperatures during the entire recovery period ranged from 18 to 25 C. Variable milfoil was harvested from each tube at 21-d and plants were placed in a drying oven at 70 C for 48 hrs and dry weights were then recorded.

This experimental design resulted in a total generation of 352 biomass values for each herbicide. At each herbicide rate and exposure tested, there were a total of 44 data points generated. Data were compared in order to determine if a response difference existed. The study was conducted using a completely randomized design with 4 replicates. Data were analyzed via Analysis of Variance (ANOVA) and means were separated using Fisher's Least Significant Difference (LSD) at the P = 0.05 level of probability.

*Results:*

Variable milfoil increased approximately 4-fold from an initial biomass of 0.09 $\pm$ 0.02 g dry wt /tube to 0.38 $\pm$ 0.06 g dry wt./tube during the 21 day pretreatment growth period. This indicates that we were applying herbicides to actively growing plants. Comparison of biomass data between the five different lakes (eleven total collection sites) indicated

minimal intra-site and inter-site variation when comparing response to similar rates and exposures of 2,4-D, triclopyr, or diquat. Of eleven individual sample sites evaluated, we found significant differences in response to a given herbicide concentration and exposure treatment in only 7/88 treatments for 2,4-D, 6/88 treatments for triclopyr, and 5/88 treatments for diquat (Table 2**)**. The few differences noted included plants that showed both greater and reduced sensitivity, and there were no clear trends between lakes or collection sites. These data likely reflect the treatment rates and short exposures that were marginal in terms of providing consistent variable milfoil control.

It should be noted that biomass data indicated significant differences ($p < 0.001$) within a given sample site (e.g. Turkey pond Site 1) and between sites (e.g. Lee's Pond Site 2 and Horseshoe pond Site 3) in response to different herbicide concentration and exposure time scenarios. This result was expected as increasing herbicide use rates and exposure times typically provides improved efficacy.

Table 2. Analysis of different treatment rates and exposures of 2,4-D, diquat, and triclopyr on variable milfoil collected from 11 discrete sites.

| **Compound** | **# Treatments showing a response difference** | **Variable milfoil from treatments sites that differed from others based on a LSD test comparing biomass when using a herbicide applied at similar treatment rates and exposures.** |
|---|---|---|
| 2,4-D<br>Liquid Amine<br>4 lbs a.i./gallon | **7 / 88** | Horseshoe Sites 1 and 2 – 1.0 mg/L for 2 hr<br>Massabesic – 1.0 mg/L for 2 hr<br>Turkey Pond Site 2 and 3 – 1.0 mg/L for 2 hr<br>Lees Pond Site 3 – 1.0 mg/L for 6 hr<br>Horseshoe Pond Site 3 – 1.0 mg/L for 6 hr |
| Triclopyr<br>Liquid Amine<br>3 lbs a.e./gallon | **6 / 88** | Horseshoe Site 2 – 1.0 mg/L for 2 hr<br>Turkey Pond Site 1 and 3 – 1.0 mg/L for 2 hr<br>Lees Pond Site 3 – 1.0 mg/L for 2hr<br>Lees Pond Site 2 – 1.0 mg/L for 6 hr<br>Lake Winn (Wolfe Bay) – 1.0 mg/L for 6 hr |
| Diquat<br>Liquid | **5 / 88** | Horseshoe Site 1 – 0.15 mg/L for 1.5 and 3 hr<br>Turkey Pond Site 1 – 0.15 mg/L for 1.5 hr |

| | | |
|---|---|---|
| 2 lbs a.i./gallon | | Lees Pond Site 2 – 0.15 mg/L for 1.5 hr<br>Lees Pond Site 3 – 0.30 mg/L for 1.5 hr |

We initiated a second trial on March 13, 2006 using the same methods as described above, but only selected concentrations and exposure periods from the first study. Water temperatures during this trial ranged from 18 to 21 C during the treatments, and 17 to 23 C during the post-treatment period. Triclopyr and 2,4-D were evaluated at 1.0 mg/L for 12 and 24 hours, and diquat was evaluated at 0.30 mg/L for 6 and 18 hours. The study included a total of 22 treatments for each sample site. Results of the second study confirmed the first, with differences between treatments noted in only 2/22 triclopyr treatments and 2/22 diquat treatments. No differences were noted for any of the 2,4-D treatments in the second study.

The documentation of hybridity in the *Myriophyllum* genus by Moody and Les (2002) has resulted in many questions being asked about possible genotype differences to herbicide management. Nonetheless, based on the weight of evidence from our studies, we conclude that different variable milfoil culture populations responded to herbicide treatments in a similar manner. While significant morphology and color differences have been noted between variable milfoil from New Hampshire and plants collected from various sites in the Southeastern U.S., all of the plants from New Hampshire were homogenous in appearance (Figure 3). Although significant intra-species variation to a given herbicide is not generally expected, there are examples of herbicide resistant plant populations in aquatics (Dayan and Netherland 2005**,** Koschnick et al. 2006)**.** Moreover, recent research by our group has evaluated the potential for different response to herbicides between populations of Eurasian and hybrid watermilfoils (*M. spicatum x M. sibiricum*) (Poovey et al. 2007, Slade et al. 2007). Given the initial objective of comparing variable milfoil population responses for a given herbicide, we did not attempt to use this data to directly compare the response of variable milfoil to the different herbicides (i.e. direct comparison of 2,4-D and triclopyr at similar rates and exposure). Comparative efficacy between compounds was the focus of all future studies.


Figure 3. The bright green variable milfoil from NH lakes had a distinct appearance compared to variable milfoil we collected from other sites. This bright green color was maintained in continuous culture.

Laboratory and mesocosm testing can not take into account all of the various physical and environmental parameters that can impact a treatment in the field (e.g. turbidity, sediment quality, plant phenology and stage of growth, epiphytic growth on the plants, water exchange characteristics, treatment timing, etc.), but it does allow us to focus on specific factors in order to determine if they can have a significant impact on efficacy. This initial work demonstrated a similar response to herbicide treatments by disjunct populations of variable milfoil collected in New Hampshire. These results suggest it is unlikely that the different variable milfoil populations within or between New Hampshire lakes would account for a high degree of variation in response to herbicide treatments.

**Comparative Efficacy Testing: Overview**

For numerous reasons, our research group has not conducted many direct comparative herbicide efficacy studies for a given species. Depending on study protocols, comparisons can often be misleading. For example, direct comparison of the rapid-acting contact product diquat against the slow-acting enzyme inhibitor fluridone is fraught with technically challenging methodological and practical issues that can render the results meaningless. Moreover, with limited research facilities, we were often limited to evaluating a single compound and species at a time. We have published numerous papers

that would allow indirect efficacy comparisons for a given species, but have not previously attempted to evaluate such a large number of products against a single species. For this project, we modified prior methods to allow for evaluation of multiple compounds and treatment scenarios against variable milfoil. One objective of this work was to delineate clear differences between compounds of a similar mode of action (or in some cases, different modes of action). For the purpose of this comparative testing, we separate the herbicide discussions into contacts, auxin-mimics, and slow-acting enzyme inhibitors. We know from experience that if you use a high enough rate and long enough exposure period of contact and auxin-mimic herbicides, you can control plants in the *Myriophyllum* genus. Unfortunately, given current use patterns, these high rate long-term exposure scenarios are often not realistic for contact and auxin-mimic products. Therefore, we specifically avoided evaluation of long-term exposures (>48 hr) for comparative research with these modes of action. In contrast, we know that short-term exposures of slow-acting enzyme inhibitors like fluridone and the ALS herbicides will only lead to short-term symptoms followed by rapid re-growth. For these products we evaluated static exposures that were maintained during the entire study. We felt this would best simulate the low-dose whole-lake use patterns that are currently practiced for plants such as hydrilla and Eurasian watermilfoil. Our goal was to give a clear picture of the potential use rates and use patterns that would provide for the most effective control of variable milfoil.

**Comparative Efficacy Testing : Contact Herbicides**

We evaluated 5 contact herbicides at various rates and exposures for activity against variable milfoil. Trials included the registered products carfentrazone, chelated copper, diquat, diquat + chelated copper, and endothall, and the EUP compound flumioxazin. While inclusion of EUP compounds required a slight alteration to the proposed schedule of work, the tradeoff was the generation of information on new classes of herbicides that may be used by the NHDES variable milfoil control program. Trials were conducted at both the CAIP facility in Gainesville, FL and the LAERF facility in Lewisville, TX. A portion of the contact herbicide work conducted at the LAERF facility was submitted and

accepted as a manuscript in the JAPM. This article entitled "Efficacy of Diquat and Carfentrazone-ethyl on Variable Leaf Milfoil" is included in Appendix 2.

*Methods:*

Variable milfoil growing in outdoor mesocosm cultures at the CAIP facility in Gainesville, FL was collected on October 4, 2005 and apical tips were planted in 150 ml culture tubes filled with nutrient amended potting soil (described above). Plants were given a 25-day pretreatment growth period and tubes were then moved to 95 L treatment containers (Figure 4). Plants were treated at selected nominal herbicide concentrations and then removed from the treatment tanks following various exposure times to determine the comparative efficacy between the compounds. Nominal herbicide use rates and exposure times are presented in Table 3**.** During the course of the herbicide exposures, water temperatures were maintained between 19 and 21 C. Following exposure, plants in the tubes were thoroughly rinsed with fresh water and then moved to a rack and placed in a 900 L tank. Water was added to the 900 L tanks to achieve two complete exchanges of volume every 24 hr for 6 d post-treatment. This was done to prevent potential for residual herbicide leaching from the plants and building to levels that could confound study results. Post-treatment water temperatures ranged from 17 to 24 C. Following a 28-day period post-treatment period, plants were harvested and shoot biomass was dried at 70 C for 48 hr.

On March 13, 2007 we initiated a second trial with selected compounds and use rates evaluated in the first study. All methods described above were repeated for the second study. Water temperatures during this trial ranged from 19 to 22 C during the treatments, and 17 to 24 C during the post-treatment period. The following treatments were chosen:

carfentrazone at 100 and 200 µg ai $L^{-1}$, diquat at 370 µg ai $L^{-1}$, flumioxazin at 400 µg ai $L^{-1}$, for 3, 6, and 12 hr. Endothall at 2500 µg ai $L^{-1}$ was evaluated at 18, 30, and 60 hr.

Figure 4. Plants in the culture tubes were moved to 95 L treatment tanks and exposed to a nominal herbicide concentration for a defined period.

Studies were conducted using a completely randomized design and each treatment was replicated 5 times. Biomass data are presented as treatment means $\pm$ 95% confidence intervals. In order to determine if any two treatments were significantly different we used paired t-test's ($\alpha = 0.05$).

Table 3. Contact herbicide treatments applied to variable milfoil for Study 1.

| Product | Treatment Rates – µg ai $L^{-1}$ | Exposure Times, Hr |
|---|---|---|
| Carfentrazone Liquid – 1 lb a.i./gal | 50, 100, 200 | 1, 3, 6, 12, 24 |
| Diquat Liquid – 2 lb a.i./gal | 90, 180, 370 | 1, 3, 6, 12, 24 |
| Diquat Copper (Komeen) Liquid – 0.8 lb a.i/gal | 370, 370, 370 250, 500, 1000 | 1, 3, 6, 12, 24 |
| Flumioxazin WP – 51% a.i | 100, 300 | 1, 3, 6, 12, 24 |
| Endothall Liquid – 4.2 lb a.i./gal | 1500, 2500 | 6, 18, 30, 48, 60 |

*Results:*

In the first study, variable milfoil increased approximately 7-fold from an initial biomass of 0.12 $\pm$ 0.03 g dry wt /tube to 0.79 $\pm$ 0.08 g dry wt./tube during the 25 day pretreatment growth period. Untreated control plants increased in mass from 0.79 to 1.68 $\pm$ 0.12 g dry wt. /tube during the post-treatment recovery period. Untreated control plants of variable milfoil were still actively growing into early December. In the second study, variable milfoil increased from 0.11 $\pm$ .05 g dry wt. /tube to 0.91 $\pm$ 0.07 g dry wt./tube during the 25 day pretreatment growth period. Untreated control plants increased in mass from 0.88 to 1.97 $\pm$ .19 g dry wt. /tube during the post-treatment recovery period. Results from both studies indicate that variable milfoil was actively growing during the herbicide exposures. Comparison of similar treatments between Studies 1 and 2 indicated no significant differences on a percent control basis ($p > 0.05$). For improved clarity in discussing the results, only data from Study 1 are presented.

Data indicate that carfentrazone was highly active at rates ranging from 50 to 200 µg ai $L^{-1}$. Treatments of 100 and 200 µg ai $L^{-1}$ at exposures of 6, 12, and 24 hr provided > 90

biomass reduction compared to untreated controls (Figure 5**).** Plants turned brown within a few days of treatment. In contrast, only the highest rate of diquat (370 µg ai $L^{-1}$**)** at the maximum exposure time of 24 hr provided control that exceeded 90%. Following diquat exposure, plants showed limited visual injury symptoms with the exception of the higher treatment rates following a 24 hr exposure. Several diquat treatments were growth inhibiting, but healthy new growth following these treatments was obvious. Neither diquat nor carfentrazone were highly active following just 1 hr of exposure; however, carfentrazone was much more active than diquat at the 3, 6, 12, and 24 hour exposure periods. The rapid activity of carfentrazone in our systems is encouraging, as it has a fairly short half-life in water due to rapid hydrolysis (Ngim and Crosby 2001, Koschnick et al. 2004**).** The short aqueous half-life and plant enzyme specificity played a role in this compound receiving a reduced risk classification by the U.S. EPA.

The addition of various rates of copper to diquat did not significantly enhance efficacy when compared to the maximum rate of diquat alone (data not shown). There was no indication of antagonism, yet the addition of copper did not increase the efficacy as we have observed on plants such as hydrilla (Sutton et al. 1972, Pennington et al. 2001**).** It should be noted that we only tested the product Komeen™ (ethylenediamine chelate), and therefore did not evaluate the numerous other chelated products that are available. Nonetheless, the lack of appreciable enhancement of efficacy at the maximum copper use rate of 1.0 mg/L, suggest that variable milfoil is not highly sensitive to copper products.

Results with diquat suggest that it has activity against variable milfoil; however, when compared to the efficacy observed against Eurasian watermilfoil (Skogerboe et al. 2006), diquat is much less active than we predicted. For example, Skogerboe et al. (2006) reported > 90% reduction in Eurasian watermilfoil biomass at use rates as low as 90 µg ai $L^{-1}$ and exposures as short as 3 to 6 hr. The use of clean epiphyte-free plants for evaluation in our system would further tend to favor diquat activity. It is probable that field plants covered in epiphytes or sediments would be even more tolerant of diquat applications. The difference in activity of diquat on variable and Eurasian milfoil is similar to recent findings by Glomski et al. (2005) who demonstrated diquat was highly

effective at low use rates and short exposures against elodea (*Elodea canadensis* Michx), but much less active on the closely related species hydrilla.

Based on our results, the maximum rate of diquat had to be maintained for 24 hr in order to achieve acceptable control of variable milfoil. By comparison, control of Eurasian watermilfoil was achieved following a few hours of exposure to 1/3 of this rate. Maintenance of extended diquat exposures in the field is confounded by dispersion from the treatment site and the affinity of diquat to bind to any negatively charged particulates in the water column. Parsons et al. (2007) observed extended exposure periods with diquat in a high clarity lake (turbidity < 1 NTU) in Washington, and attributed the level of target plant control achieved to the ability to maintain these residues. In systems with higher turbidity, diquat efficacy would likely be compromised on a moderately susceptible species such as variable milfoil (Poovey and Getsinger 2002). The low levels of turbidity in many New Hampshire lakes may explain why diquat has shown some degree of effectiveness for control of variable milfoil.

Evaluation of the EUP compound flumioxazin suggests that variable milfoil responds to this herbicide in a manner very similar to carfentrazone (Figure 6). This is not surprising as these herbicides share a common mode of action (inhibition of the same enzyme protoporphyrinogen oxidase). Comparison of the 100 μg ai $L^{-1}$ treatments indicate that carfentrazone is more active on variable milfoil than flumioxazin. Nonetheless, head to head comparisons of these products are premature, as label use rates and product costs will ultimately determine the most cost-effective option between these two herbicides. It should be noted that both of these protox inhibitors provided superior control when compared to diquat. At this point in time, carfentrazone is the only protox inhibitor that has a US EPA Section 3 label for aquatic use.

Endothall trials provided evidence of activity, but variable milfoil control was quite limited when compared to the other contact products we evaluated (Figure 6). As was noted with diquat, endothall has much greater activity on Eurasian watermilfoil compared to variable milfoil (Netherland et al. 1991). While increasing the use rate or significantly

extending the exposure period would likely enhance the efficacy, the requirement for high use rates and extended exposures typically indicates a product that will provide only marginal control in many situations. It is interesting to note that when we evaluated endothall on a variable milfoil population from Georgia, we noted much greater efficacy than was observed for plants from New Hampshire. The distinct morphological (and potentially genetic) differences between the variable milfoil populations in New Hampshire and the southern US may explain some of the differences we observed in response to herbicides.

The high level of carfentrazone activity on variable milfoil was somewhat unexpected given the generally weak response demonstrated on other milfoil species (Glomski et al. 2006, Gray et al. 2007). It should be noted that our exposures were conducted under a pH of ~6.5, while other milfoil species were likely exposed when the aqueous pH was much greater (8.0 to 9.0). Subsequent evaluations of both carfentrazone and flumioxazin on other submersed species indicate enhanced activity of these compounds under low pH conditions. Both of these compounds are subject to fairly rapid hydrolysis, and product half-lives for flumioxazin can be as short as a few minutes at a pH above 8. The increased residue longevity under more acidic conditions (pH < 7) may enhance the activity of these products on variable milfoil. This suggests that prevailing water quality conditions in New Hampshire may favor a compound such as carfentrazone that shows strong activity in lower pH waters. Our data indicate that both carfentrazone and flumioxazin have strong potential for use as contact herbicides for control of variable milfoil in New Hampshire.

The repeat trial initiated in March 2006 comparing efficacy results from selected herbicide rates and exposures to results obtained in the first trial indicated there were no significant differences in the treatment outcomes on a percent control basis. Results confirmed that activity was similar for studies conducted on young actively growing variable milfoil.

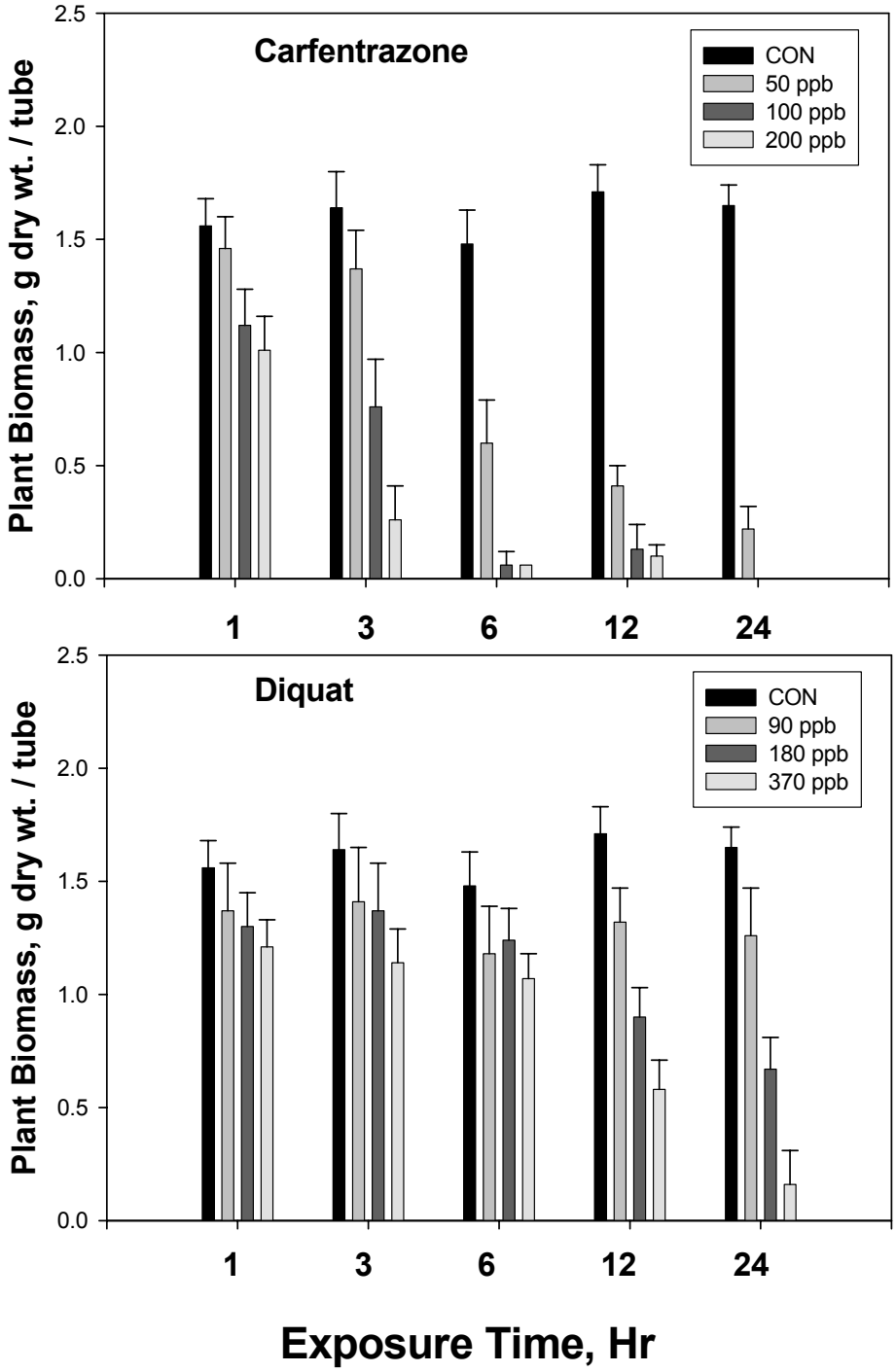


Figure 5. Dry biomass of variable milfoil following exposure to various concentrations and exposures of carfentrazone and diquat. Each bar represents the average of 5 treatments with 95% confidence intervals.

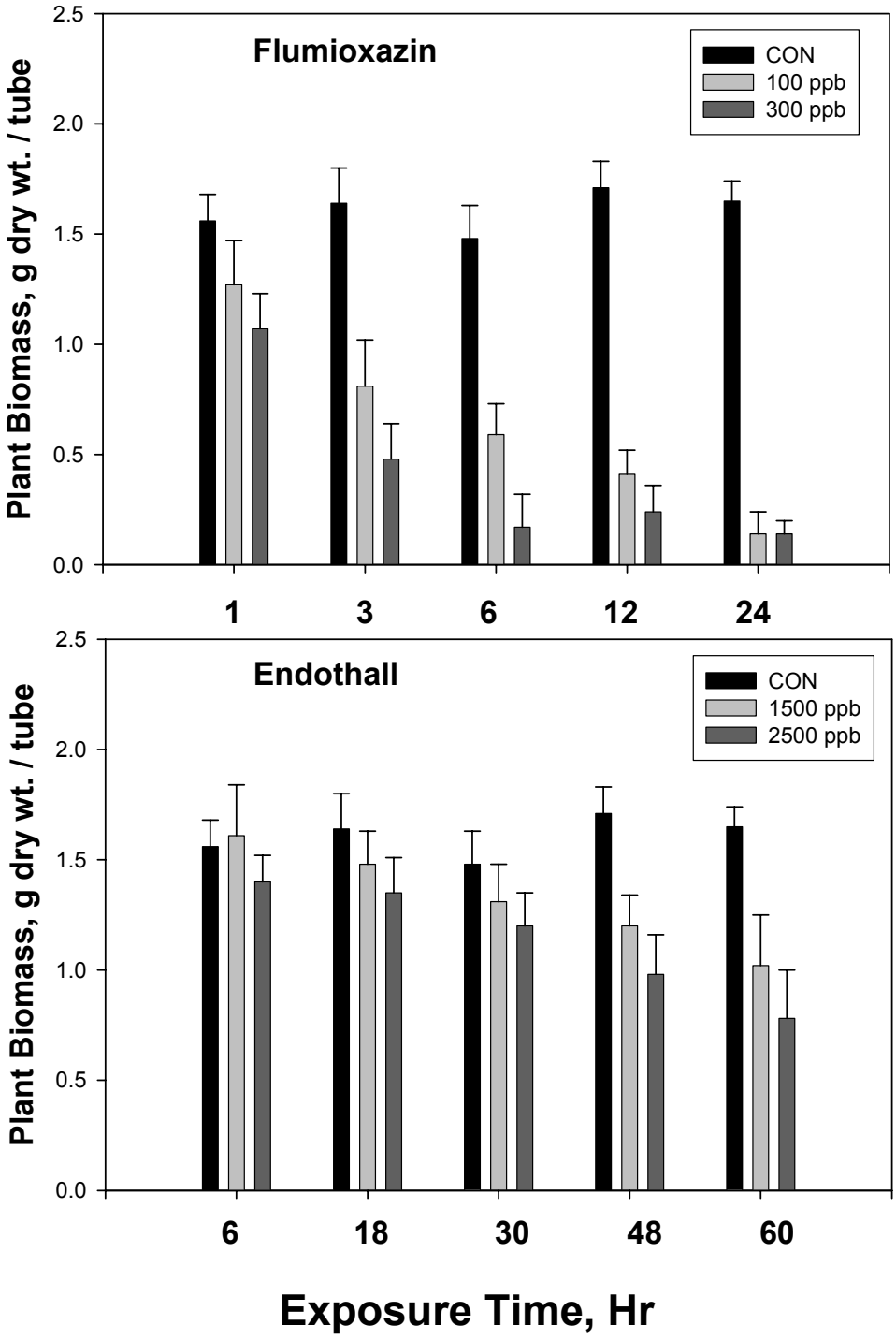


Figure 6 Dry biomass of variable milfoil following exposure to various concentrations and exposures of flumioxazin and endothall. Each bar represents the average of 5 treatments with 95% confidence intervals.

**Comparative Efficacy Testing : Auxin-Mimic Herbicides**

We evaluated 4 auxin-mimic herbicide formulations at various rates and exposures for activity against variable milfoil. Trials included the registered products 2,4-D butoxyethylester (BEE), 2,4-D amine, and triclopyr amine. We also evaluated the EUP compound quinclorac. Trials were conducted at both the CAIP facility in Gainesville, FL and the LAERF facility in Lewisville, TX. Initial pilot studies indicated that use rates of 1.0 and 2.0 mg/L and extended exposures (48, 72, and 96 hrs) of 2,4-D and triclopyr consistently provide 97 to 100% control of the newly established variable milfoil. The auxin herbicide work we present was conducted in conjunction with the contact herbicide trials described above. Therefore, timing, water temperatures, and other methods are similar to the methods used to evaluate the contact products. We did test different use rates and exposure times for the auxin mimics. For improved clarity we have chosen to present these systemic growth-regulating herbicides as a separate section. Due to the similar experimental design and the fact that studies were conducted at the same time, direct comparison of the contact herbicide data with the auxin-mimic data is possible. We will reference some of the contact herbicide data in this section.

*Methods:*

The auxin-mimic herbicide trials were conducted in conjunction with the contact herbicide trials and methods are described in the previous section. Nominal herbicide use rates and exposure times for the auxin-mimics are presented in Table 4**.** For the second trial, the following treatments were chosen: 2,4-D BEE, 2,4-D amine, triclopyr amine and quinclorac at 500 and 1500 µg ai $L^{-1}$ for 3, 6, and 24 hr.

For this evaluation of the 2,4-D BEE granular, we added the product to the treatment tanks to insure there was no physical contact of the granules with the plants or the sediments. This allowed us to evaluate the impact of the aqueous residues alone, and avoided confounding issues due to concentrating granules on the plants or on the sediment at the rootcrown. We collected water samples for both the liquid and granular 2,4-D and triclopyr treatments at 1, 3, 6, 12, and 24 hours. Water samples were analyzed

via enzyme-linked immunoassay kits for 2,4-D and triclopyr (Strategic Diagnostics Inc.). The 2,4-D samples were analyzed at the UF CAIP and triclopyr samples were analyzed by the SePRO Corporation Laboratory in Whitakers, North Carolina. The data presented are combined results from the October 2005 and March 2006 trials.

Studies were conducted using a completely randomized design and each treatment was replicated 5 times. Biomass data are presented as treatment means $\pm$ 95% confidence intervals. In order to determine if any two treatments were significantly different we used paired t-test's ($\alpha = 0.05$).

Table 4. Auxin-mimic herbicide treatments applied to variable milfoil.

| **Product** | **Treatment Rates – µg ai $L^{-1}$** | **Exposure Times, Hr** |
|---|---|---|
| 2,4-D BEE<br>Max use rate – 4000 µg ai $L^{-1}$ | 500, 1000, 1500, and 2000 | 1, 3, 6, 12, 24 |
| 2,4-D Amine<br>Max use rate – 4000 µg ai $L^{-1}$ | 500, 1000, 1500, and 2000 | 1, 3, 6, 12, 24 |
| Triclopyr<br>Max use rate – 2500 µg ai $L^{-1}$ | 500, 1000, 1500, and 2000 | 1, 3, 6, 12, 24 |
| Quinclorac | 500, 1000, 1500, and 2000 | 1,3, 6, 12, 24 |

*Results:*

As reported in the contact herbicide section, variable milfoil was actively growing during prior to the herbicide exposures and during the post-treatment evaluation period. Actual biomass values for the untreated controls were reported in the previous results section. Comparison of similar treatments between Studies 1 and 2 indicated no significant differences on a percent control basis ($p > 0.05$). For improved clarity in discussing the efficacy results, only data from Study 1 are presented.

Residue analyses from both trials indicated that nominal concentrations of triclopyr and 2,4-D were essentially achieved at 1 hr post-treatment and maintained through the 24-

hour exposure period (Table 5). In contrast, 2,4-D residues following application of the BEE formulation increased through the 24-hr exposure period and theoretical target concentrations were never achieved (Table 5). Initial inspection of this data would suggest problems in direct comparison of treatments due to the lower residues experienced following the 2,4-D BEE applications.

Table 5. Percent recovery of triclopyr and 2,4-D residues (± 1 S.D.) from treatment tanks following application of liquid amine and granular ester formulations.

| **Compound** | **% of Target Active Ingredient Recovered** | | | | |
|---|---|---|---|---|---|
| | **1 Hr** | **3 Hr** | **6 Hr** | **12 Hr** | **24 Hr** |
| 2,4-D Liquid Amine | 97 (5) | 95 (8) | 98 (6) | 95 (4) | 96 (7) |
| Triclopyr Liquid Amine | 94 (5) | 94 (9) | 97 (8) | 93 (5) | 95 (5) |
| 2,4-D BEE granular ester | 16 (8) | 24 (10) | 38 (9) | 58 (11) | 86 (6) |

Treatments resulted in bending and twisting of the shoot meristems within the first 24 hours of treatment. While this epinasty was observed with all compounds and use rates, this did not necessarily translate to control of the plants. For example, while the 1-hr exposures resulted in strong initial injury symptoms, only the 2,4-D BEE treatment resulted in a significant biomass reduction compared to the initial biomass level of 0.79 g/tube. Symptoms were essentially indistinguishable between 2,4-D, triclopyr, and quinclorac at all rates and exposures tested.

Study results clearly indicate that 2,4-D BEE was the most effective compound under the majority of treatment rates and exposure scenarios we tested (Figures 7 and 8). This result was unexpected for two reasons. First, the study was designed to prevent the influence of a high concentration of 2,4-D at the base of the plants and therefore we know that all exposure occurred only through shoot uptake from the surrounding water column. Second, the residues of 2,4-D following the BEE treatments were consistently and

significantly lower than the residues of the liquid amine treatments. Prior CET studies would suggest that the lower concentration treatments should result in reduced efficacy, especially with the shorter exposure periods.

The amine formulations of 2,4-D and triclopyr produced similar results with triclopyr showing enhanced efficacy at the higher concentrations and exposures. The higher use rate and longer exposures suggest that either triclopyr or 2,4-D amine can provide good control of variable milfoil in situations where the residues do not readily disperse from the treatment site. The quinclorac treatments provided biomass reduction; however, variable milfoil regrowth was evident following many of the treatments. Field use patterns with quinclorac for hydrilla control suggest that this product may require longer exposure periods (several days) in order to provide desired levels of control. We did not test quinclorac under longer exposure regimes on variable milfoil.

The repeat trial initiated in March 2006 comparing efficacy results from selected herbicide rates and exposures to results obtained in the first trial indicated there were no significant differences in the treatment outcomes on a percent control basis. Results confirm that activity was similar for studies conducted on young actively growing variable milfoil.

While there has been much speculation regarding the relation between efficacy of granules and placement of product near the rootcrown of the plant, these results suggest other factors influenced the efficacy of the BEE granules. It has been documented that the ester form of 2,4-D is generally more toxic to fish and invertebrates when directly compared to the amine formulation (USEPA 2005, WSDOE 2001). The differences between the toxicological endpoints of the two products are discussed in the recent re-registration eligibility decision for 2,4-D (USEPA 2005). The potential for increased activity of the BEE formulation on submersed plants has not been well characterized.

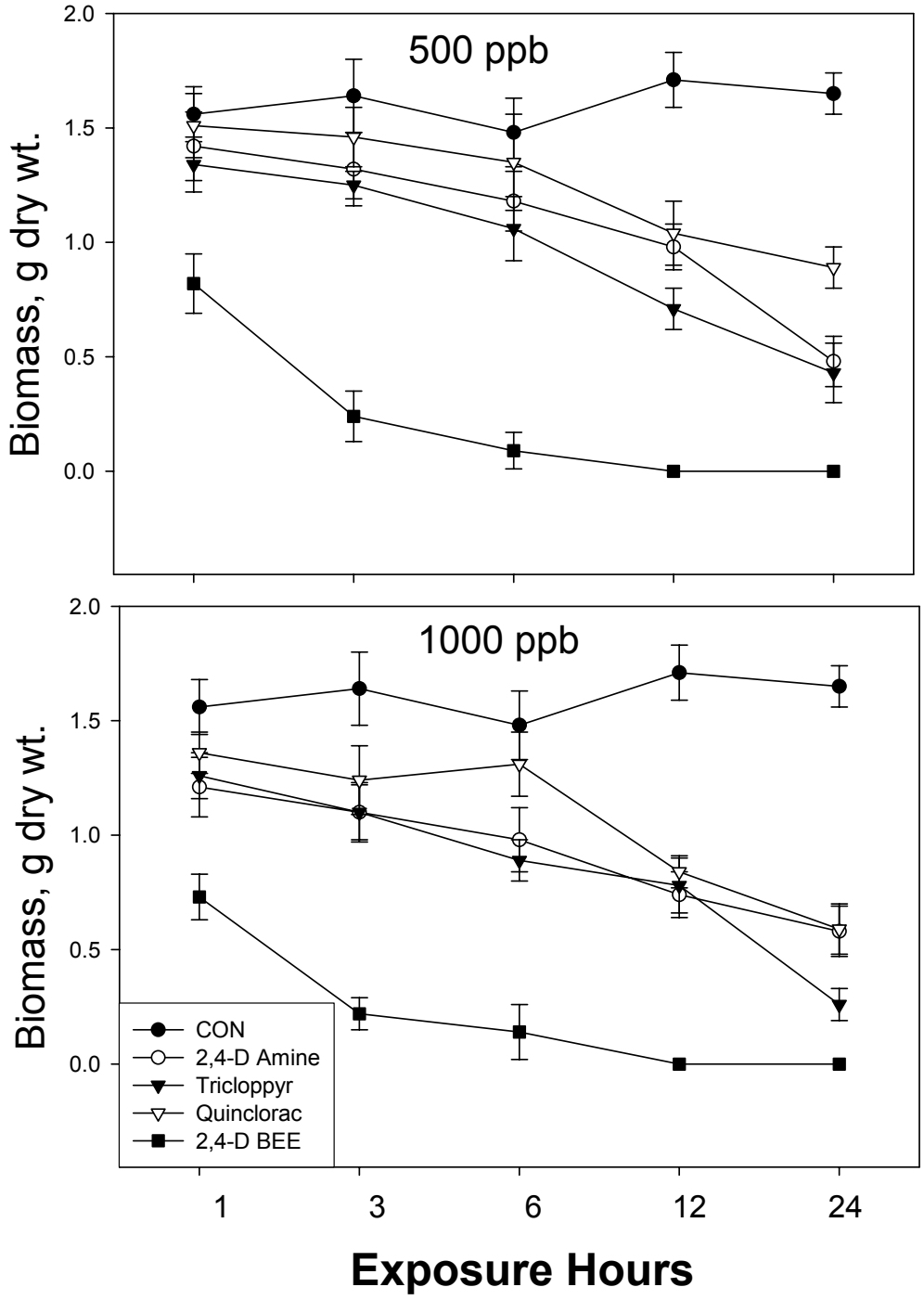


Figure 7. Response of variable milfoil to different concentrations and exposures of herbicides. Each point represents the average of 5 replicate treatments ± 95% C.I.

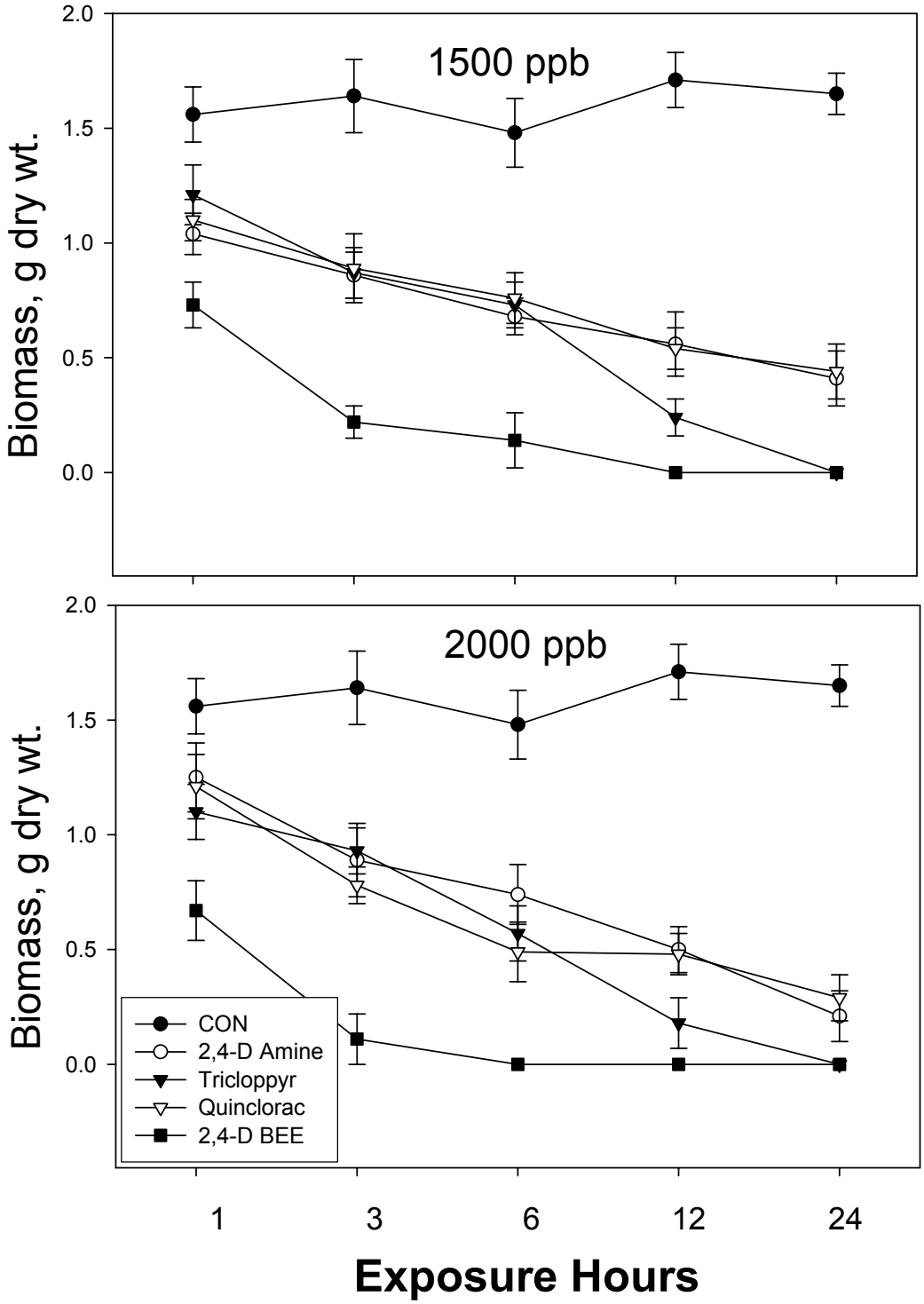


Figure 8. Response of variable milfoil to different concentrations and exposures of herbicides. Each point represents the average of 5 replicate treatments ± 95% C.I.

From a management perspective, the magnitude of the difference in response of variable milfoil to the ester and amine formulations in our studies indicate the ester provides a clear efficacy advantage when controlling this species in low alkalinity waters. It should be noted that using similar methodology, we were not able to identify clear differences in the response of Eurasian watermilfoil to the ester versus the amine formulations (Netherland et al. 2007). To date, out data suggest the combination of an inherent species response and low alkalinity/pH are responsible for the increased level of variable watermilfoil sensitivity to 2,4-D ester. The results from this study indicate that 2,4-D BEE impacts on variable milfoil prove to be an exception to accepted CET relationships developed for many other herbicides and plant species.

In summary, all of the auxin-mimic compounds showed potential to provide control of newly established and actively growing variable milfoil. Efficacy of 2,4-D amine and triclopyr amine improved as exposure periods were extended. Quinclorac, while active on variable milfoil, will require further testing prior to making recommendations regarding use rates and a use pattern. Based on these results, we recommended further testing of 2,4-D BEE, triclopyr amine, and a prototype triclopyr granule on mature variable milfoil.

**Evaluation of 2,4-D BEE, and Triclopyr on Mature Variable Milfoil**

Initial efficacy studies were conducted on smaller and newly established variable milfoil plants. To validate these results, we evaluated the impact of selected herbicides on larger more established plants. We chose to evaluate 2,4-D BEE, triclopyr amine, and a prototype granular amine formulation of triclopyr.

*Methods:*

Variable milfoil plants that had been growing in culture for over 15 months at the UF CAIP were selected for this study. The plants were growing in 4 L pots and each pot

contained a large rootcrown with numerous shoots of variable milfoil (Figure 9). On May 2, 2006, selected plants were transferred to 95 L treatment tanks. Treatment rates and exposure times are noted in Table 6**.** Water temperatures at the time of exposure ranged from 22 to 24 C. Following the designated exposure period, pots were removed from the treatment tanks, thoroughly rinsed, and then placed in a 900 L recovery tank. Water was added to the 900 L tanks to achieve two complete exchanges of volume every 24 hr for 6 d post-treatment. This was done to prevent potential for residual herbicide leaching from the plants and building to levels that could confound study results. Post-treatment water temperatures ranged from 20 to 28 C. Following a 70-day post-treatment period, plants were harvested and shoot biomass was dried at 70 C for 48 hr.

For this study, the granular products were sprinkled evenly across the treatment tanks. In contrast to the study noted above, we did not take any measure to prevent granules from landing on plant tissue or on the sediments. We collected water samples for both the

Figure 9. Established variable milfoil plants with extensive root systems and numerous shoots emerging from the rootcrown.

liquid and granular 2,4-D, and the granular triclopyr treatments at 6, 12, 24, and 48 hours. Water samples were analyzed via enzyme-linked immunoassay kits for 2,4-D and triclopyr. The 2,4-D samples were analyzed at the UF CAIP and triclopyr samples were analyzed by the SePRO Corporation Laboratory in Whitakers, North Carolina.

This study was repeated on September 12, 2006. Water temperatures at the time of exposure ranged from 21 to 24 C. Post-treatment water temperatures ranged from 19 to 26 C. All treatment rates, exposures, and other methods were repeated as stated above.

Studies were conducted using a completely randomized design and each treatment was replicated 4 times. There were no significant differences in the biomass or residue data between the two studies and therefore data have been combined for presentation. Biomass data are presented as treatment means $\pm$ 95% confidence intervals. In order to determine if any two treatments were significantly different we used paired t-test's ($\alpha$ = 0.05)

Table 6. Herbicide treatments, rates, and exposures applied for evaluating efficacy against mature variable milfoil.

| **Product** | **Treatment Rates – µg ai $L^{-1}$** | **Exposure Times, Hr** |
|---|---|---|
| 2,4-D BEE<br>*Granular ester 19.1% a.e.* | 500 and 1500 | 6, 12, 24, 48 |
| 2,4-D Amine<br>*Liquid amine 4 lb a.i./gal* | 500 and 1500 | 6, 12, 24, 48 |
| Triclopyr Granular<br>*Granular amine 10.1% a.e.* | 500 and 1500 | 6, 12, 24, 48 |

*Results:*

The initial biomass of the variable milfoil for Study 1 was 27.9 $\pm$ 4.3 g dry wt. / pot. The untreated controls increased to 39.4 $\pm$ 5.1 g dry wt./pot during the 70-d post-treatment period. Initial biomass for study 2 was 34.1 $\pm$ 3.1 g dry wt./pot and increased to 42.8 $\pm$ 4.9 g dry wt. / pot. While these growth rates were slower than those observed in prior

studies, the increase in biomass does demonstrate that variable milfoil was actively growing at the time of treatment.

Residue analyses indicated that nominal concentrations of 2,4-D were maintained throughout the exposure following the amine treatment (Table 7). Residues following the 2,4-D BEE and triclopyr granular applications steadily increased through the first 24 hours and then slightly increased from 24 to 48 hours (Table 7). As noted in the previous trial, while the initial target concentrations were similar for all treatments, the actual residues achieved at various exposure times were different. While comparison of different aqueous residues may seem problematic in these small-scale studies, the residues patterns likely reflect inherent differences between liquid and granular formulations following field application. Water samples collected in the recovery tank at 48 hours following the addition of pots from the treated tanks did not result in the recovery of 2,4-D or triclopyr residues.

Table 7. Percent recovery of triclopyr and 2,4-D residues ($\pm$ 1 S.D.) from treatment tanks following application of liquid amine and granular ester formulations.

| **Compound** | **% of Target Active Ingredient Recovered** | | | | |
|---|---|---|---|---|---|
| | **6 Hr** | **12 Hr** | **24 Hr** | **48 Hr** | **Recovery Tank (48 hr)** |
| 2,4-D Amine | 93 (6) | 96 (8) | 95 (2) | 94 (5) | 0 |
| 2,4-D BEE | 27 (8) | 52 (11) | 79 (7) | 91 (5) | 0 |
| Triclopyr Granular | 39 (7) | 64 (12) | 87 (9) | 96 (5) | 0 |

Treated plants showed strong evidence of epinasty by 24 hr after treatment. As noted above, similar symptoms did not always translate to equivalent efficacy. Study results clearly indicate that 2,4-D BEE remained the most effective compound following all exposure periods of 24 hr and less (Figures 10 and 11). Healthy regrowth was noted in all pots treated with 2,4-D amine and granular triclopyr at exposure periods of 24 hr and less. In contrast, all 1500 µg ai $L^{-1}$ treatments were highly effective when given a 48-

hour exposure period (Figure 10). Efficacy differences between the 2,4-D BEE and triclopyr amine granule further suggest that the ester formulation is responsible for the enhanced efficacy against variable milfoil. It should be noted that a granular formulation of triclopyr has recently been registered for aquatic use (Renovate OTF); however, it should be noted the formulation we evaluated was an early prototype of this formulation.

Results indicate that all of these products can provide control of mature variable milfoil under favorable concentration and exposure time scenarios; however, the ester formulation of 2,4-D would likely provide the best efficacy under the widest range of treatment situations, especially where short exposure times might be expected.

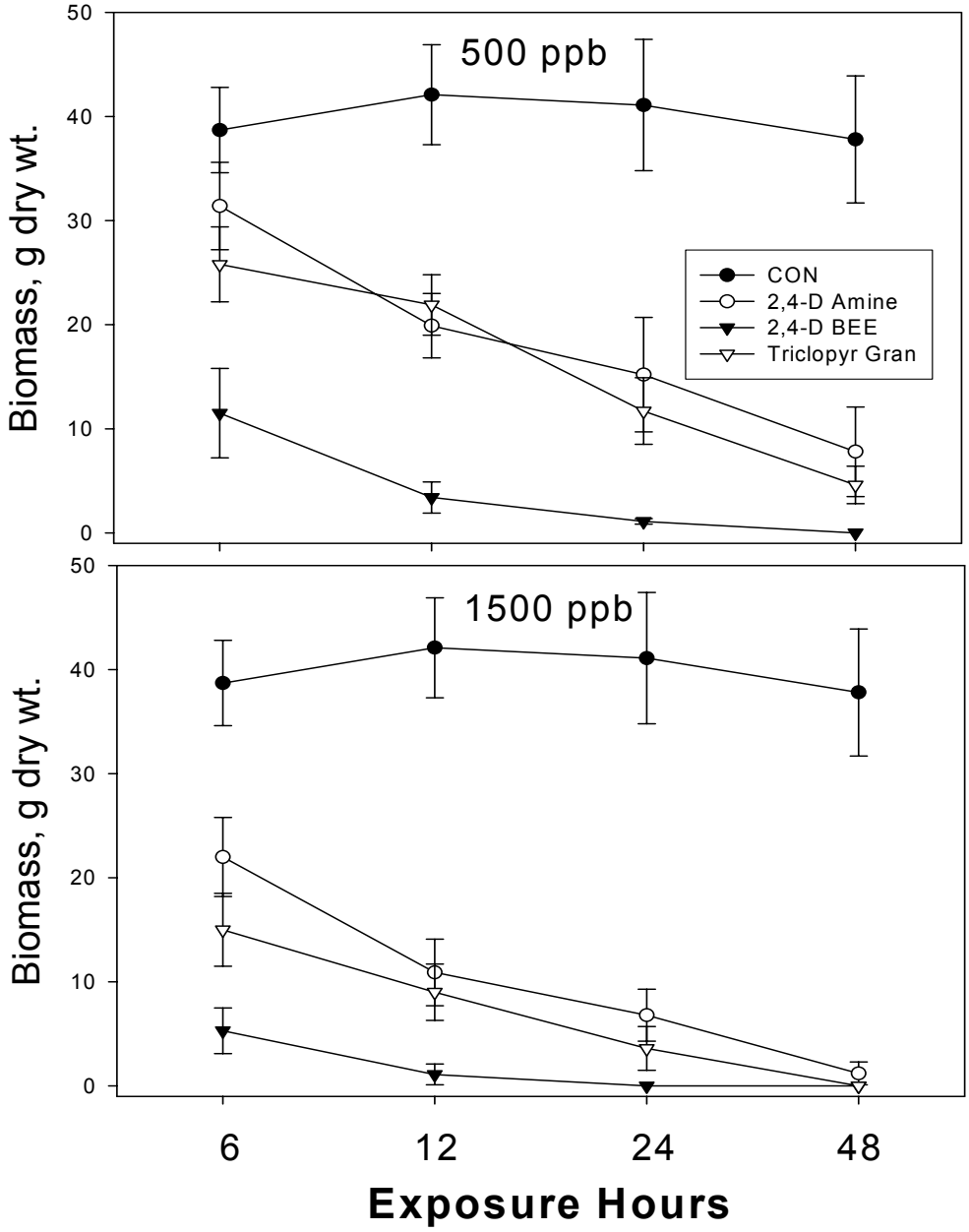


Figure 10. Biomass response of established variable milfoil to various concentrations and exposures of 2,4-D ester, 2,4-D amine, and triclopyr amine. Each symbol represent the average of 5 replicate treatments (+95% confidence intervals).

**Triclopyr Gran** **CON** **2,4-D BEE**

Figure 11. Response of variable milfoil to triclopyr and 2,4-D BEE treatments.

**Evaluation of Slow Acting Enzyme Inhibitors**

We evaluated four herbicides that impact the plant-specific enzymes phytoene desaturase (fluridone) and acetolactate synthase (penoxsulam, bispyribac, and imazamox). Fluridone and penoxsulam are currently registered for aquatic use and imazamox and bispyribac are being evaluated under Experimental Use Permits for aquatic use. While there is significant literature regarding the activity of fluridone on submersed plants, information on the activity of ALS inhibitors against submersed plants remains quite limited.

Although penoxsulam, imazamox, and bispyribac sodium are classified into three different herbicide families (triazolopyrimidines, imidazolinones, and

pyrimidinylthiobenzoates, respectively), all three impact the same plant enzyme. Despite sharing common modes of action, ALS herbicides are known to have quite different effects on plant species. Selectivity in all families appears to be rate dependent. Slight changes in the molecular structure of ALS-inhibiting herbicides greatly affect the potency and weed spectrum (Ren et al. 2000). This, along with low use rates and low toxicity to mammals and fauna has lead to registration of more than 50 ALS inhibiting herbicides for weed control in a variety of terrestrial weed management programs (Heap 2005). The toxicology profiles of most ALS inhibitors under evaluation in aquatics will likely allow immediate consumptive use of the water (potable uses, fishing, or swimming)

The concept of low dose and extended exposure periods is quite familiar with fluridone, and prior and recent research data suggests that the ALS inhibitors will act in a similar manner (Netherland et al. 1993, Nelson et al. 1993, Langeland and LaRoche 1992). Nelson et al. 1993 found that a broad range of bensulfuron-methyl (ALS inhibitor) use rates stopped the growth of Eurasian watermilfoil, yet an extended exposure period was critical to achieving optimal control. Extended exposure requirements for products like fluridone and ALS inhibitors will likely dictate that these herbicides are used for low dose whole-lake applications, or in isolated coves that have limited water exchange with the main body of the lake. The efficacy and selectivity of fluridone as a whole-lake treatment for Eurasian watermilfoil is well documented (Getsinger et al. 2002a, Getsinger et al. 2002b).

Studies with the ALS inhibitors were conducted at the LAERF in Texas, and the article submitted for journal publication is included in Appendix 3. In summary, the results of this work suggest that fluridone and penoxsulam are quite active on variable milfoil at similar use rates, while imazamox and bispyribac were not effective at the use rates evaluated (Figure 12). Further field evaluations with fluridone and penoxsulam would be warranted based on the data collected to date. It should be noted that both fluridone and penoxsulam will show the best activity under conditions of active growth and low biomass. Late season treatments or treatment of plants that have already formed extensive canopies are not recommended. The decision to implement whole-lake

management with these compounds requires that numerous factors be considered including: 1) the potential for flow to reduce residues below phytotoxic levels; 2) potential impact on both submersed and emergent non-target plants; 3) slow activity of enzyme inhibitors will not result in immediate reduction in plant mass; and 4) the maintenance of low but phytotoxic residues may require multiple applications.

Fluridone

Penoxsulam

Bispyribac

Imazamox

Figure 12. Response of variable milfoil to selected rates and continuous exposures of four slow acting enzyme inhibiting herbicides.

**The Impact of Water Temperature on Herbicide Efficacy**

Numerous environmental factors (e.g. pH, turbidity, temperature, water flow) can impact the efficacy of aquatic herbicides. One factor that has received limited research attention is the impact of water temperature on efficacy. This is a pertinent question as low plant biomass and active growth early in the spring favor treatment, but this must be balanced against treating under cool water conditions. Over time, various herbicide labels have recommended that water temperatures be above 16 C (60 F) prior to treatment. Previous research with the contact herbicides diquat and endothall on curlyleaf pondweed (*Potamogeton crispus* L.) demonstrated that lower water temperature could negatively impact efficacy (Netherland et al 2001). Nonetheless, the information generated from this work supported operational recommendations in the upper Midwest U.S. that led to early-season (March to early April) cool water treatments (> 13 C) of curlyleaf pondweed with endothall (Poovey et al 2002). These early treatments disrupt the life cycle of the plant by preventing turion production. While variable milfoil does not produce vegetative propagules such as turions or tubers, early season applications could have several benefits including: 1) targeting reduced plant biomass and storage reserves early in the season; 2) treatment of actively growing young plants; 3) reduced accumulation of epiphytic growth and sediments on the plant tissue; and 4) reduced interference of the treatment with recreational utilization. Any early season treatment strategy must be balanced against the higher water flow rates that are characteristic of spring in New England.

Temperature studies were conducted at both the UF CAIP and the LAERF in Texas. Work conducted at the LAERF has been submitted for journal publication and is included in Appendix 4. This study evaluated the efficacy of carfentrazone and 2,4-D BEE at temperatures of 13, 16, 19, and 22 C. In summary the results of this work suggest that water temperature did not impact the efficacy of either carfentrazone or 2,4-D. Studies conducted at the UF CAIP are described below.

*Methods:*

On March 6, 2006, variable milfoil culture plants (similar to the mature plants described above) that had been established in 4 L containers were placed in 95 L treatment tanks located in greenhouses maintained at temperatures of 15, 20, and 25 C. Prior to moving these plants, ambient temperatures in the culture tanks ranged from 17 to 22 C. Plants were given a 3 d acclimation period in the greenhouse. Treatments included carfentrazone at 150 µg ai $L^{-1}$, and triclopyr and 2,4-D amine at 1000 µg ai $L^{-1}$ for exposure periods of 3, 6, 9, 16, and 24 hours. Following herbicide exposure, plants were placed outdoors in a 900 L grow-out tank. Water was added to the 900 L tank to achieve two complete exchanges of volume every 24 hr for 6 d post-treatment. This was done to prevent potential for residual herbicide leaching from the plants and building to levels that could confound study results. Ambient water temperatures in the recovery tanks ranged from 18 to 25 C during the 5-week recovery period. Plants were harvested at 5 weeks after treatment and shoot biomass was dried at 70 C for 48 hr.

Studies were conducted using a randomized block design and each treatment was replicated 5 times. Biomass data are presented as treatment means $\pm$ 95% confidence intervals. In order to determine if any two treatments were significantly different we used paired t-test's ($\alpha = 0.05$).

*Results:*

The initial biomass of the variable milfoil was 21.1 $\pm$ 2.8 g dry wt. / pot. Growth of the untreated controls exposed to 15, 20, and 25 C did not differ and biomass increased to an average of 32.5 $\pm$ 3.4 g dry wt./pot during the 35-d post-treatment period. The increase in biomass indicates that variable milfoil was actively growing at the time of treatment.

Results of this study showed that water temperature did not impact the efficacy of any of the herbicides evaluated (Table 8). Carfentrazone was the most effective treatment especially when comparing the shorter exposure periods. Variable milfoil biomass was reduced by greater than 80 % compared to untreated controls following all carfentrazone

treatments. As exposure periods were increased the efficacy differences between carfentrazone, 2,4-D amine, and triclopyr were less pronounced.

These results suggest that as long as the plants are actively growing, water temperature should not have a strong impact on treatment efficacy. The enhanced efficacy of carfentrazone compared to the 2,4-D and triclopyr amine formulations indicate that carfentrazone should be considered for field evaluation.

Table 8. Percent control (± 95% confidence intervals) of variable milfoil at 5 weeks following treatment with carfentrazone, 2,4-D, and triclopyr at various exposure periods at water temperatures of 15, 20, and 25 C.

| **Compound** | **Exposure Temp** | **% control 3 hr Exp.** | **% control 6 hr Exp.** | **% control 9 hr Exp** | **% control 16 hr Exp** | **% control 24 hr Exp** |
|---|---|---|---|---|---|---|
| | | | | | | |
| Carfentrazone 150 µg ai $L^{-1}$ | **15** | 83 (6) | 85 (4) | 88 (6) | 98 (2) | 100 |
| Carfentrazone 150 µg ai $L^{-1}$ | **20** | 87 (3) | 83 (6) | 88 (4) | 95 (3) | 98 (2) |
| Carfentrazone 150 µg ai $L^{-1}$ | **25** | 82 (6) | 82 (5) | 89 (5) | 96 (3) | 97 (3) |
| | | | | | | |
| 2,4-D Amine 1000 µg ai $L^{-1}$ | **15** | 18 (5) | 37 (4) | 55 (11) | 71 (6) | 82 (4) |
| 2,4-D Amine 1000 µg ai $L^{-1}$ | **20** | 22 (5) | 40 (6) | 52 (8) | 77 (10) | 87 (9) |
| 2,4-D Amine 1000 µg ai $L^{-1}$ | **25** | 25 (7) | 42 (9) | 60 (12) | 70 (8) | 84 (7) |
| | | | | | | |
| Triclopyr 1000 µg ai $L^{-1}$ | **15** | 21 (6) | 34 (8) | 59 (5) | 79 (4) | 93 (6) |
| Triclopry 1000 µg ai $L^{-1}$ | **20** | 23 (4) | 45 (8) | 67 (7) | 85 (9) | 91 ( 4) |
| Triclopyr 1000 µg ai $L^{-1}$ | **25** | 27 (7) | 41 (9) | 70 (9) | 88 (9) | 90 (6) |

**Field Validation of the Activity of Carfentrazone and 2,4-D BEE on Variable Milfoil in North Carolina**

The work proposed for this research effort focused on laboratory and mesocosm efforts. Nonetheless, our research group did take advantage of the opportunity to coordinate with researchers from North Carolina State University to evaluate the efficacy of carfentrazone (applied as Stingray™) and triclopyr (applied as Renovate™) in North Carolina waterbodies infested with variable milfoil (Figure 13). The attached observations from Dr. Rob Richardson summarize the treatments applied and visual observation of results from these field trials.

Frye Pond – 2 Acres- treated approximately ¼ acre
Frye Pond, Moore Co. – Stingray @ 200ppb applied 8 June, 2006: 2 WAT = 98% control; 4 WAT = 100% control

Church Pond – 1.38 Acres - treated 0.7 acres
Church Pond, Moore Co. – Stingray @ 100ppb applied 10 August, 2006: 2 WAT = 80 % control; 4 WAT = 88% control; 6 WAT = 83% control – regrowth noted

Hoke Co. Pond – 5.67 acres - treated 1.14 acres
Hoke Co. Pond – Renovate @ 1.0 ppm applied 23 June, 2006: 2 WAT = 90% control; 4 WAT = 100% control; 8 WAT = 100% control

Pender Co. Pond 1/3 acre - treated 0.15 acres
Pender Co. Pond - Renovate @ 0.5 ppm applied 24 July, 2006: 3 WAT = 90% control; 6 WAT = 95% control; 8 WAT = 95% control

These treatments confirm that carfentrazone is highly active on variable milfoil growing under field conditions. Moreover, the low rates and extended exposures of triclopyr were quite effective in controlling variable milfoil in ponds. The variable milfoil problems in New Hampshire tend to be represented by localized infestations in larger water bodies, and therefore pond trials in North Carolina may not be directly applicable due to the ability to achieve extended exposure periods. Nonetheless, these initial field trials do give us increased confidence in making recommendations for a new product like carfentrazone.

Figure 13. Variable milfoil infestation on a small pond in North Carolina.

It is worth noting that the variable milfoil from New Hampshire sites has a very distinct morphology and color compared to plants observed in North Carolina and other parts of the Southeastern U.S. While the color differences have been related to water temperatures, the morphology and color differences were sustained despite plants being grown in similar culture conditions for over 2 years. Despite the significant morphological differences, limited herbicide testing at the mesocosm scale did not result in any notable differences between variable milfoil from New Hampshire and plants collected from Georgia or Florida.

**Evaluation of 2,4-D and Triclopyr for Control of Emerged Variable Milfoil**

*Background:*

During the course of our research we discovered that upon removal of pots containing variable milfoil from submersed culture tanks, small shoots would extend from the rootcrown and become established as an emergent growth form (Figure 14). These emerged forms remained small and succulent, but would persist for months as long as we

maintained a routine watering regime (similar to watering requirements for a terrestrial plant). Upon re-submerging the pots containing emergent variable milfoil, the plants would quickly (less than one week) transition to the submersed form, elongate and start growing to the water surface. This suggested that emergent forms of variable milfoil could serve as a source of re-infestation in situations where water levels have dropped and plants remain established along the exposed banks.

We tested the sensitivity of the emergent form of variable milfoil to 2,4-D and triclopyr amine. Both of these liquid products have shown good efficacy on the submersed form of variable milfoil, and both products contain label directions for controlling emergent and floating aquatic plants. We also wanted to test the sensitivity of the emerged form of the variable milfoil to an aqueous exposure of 2,4-D and triclopyr immediately after flooding.

*Methods:*

Pots containing variable milfoil were removed from submersed culture and were exposed to drying conditions outdoors. The pots were either 1 L or 3.8 L in size. Emerged forms of variable milfoil were allowed to establish in the pots and were watered once a week in a manner similar to that used for maintaining a typical houseplant (no standing water in the pots). The emerged forms remained small and close to the soil level. The plants had a waxy feel that suggested formation of a cuticle was providing protection from desiccation. Plants were maintained in an emergent form for a minimum of 1-month prior to use in studies. Evaluations were conducted in August 2006 and October 2006.

For both the 2,4-D and triclopyr treatments we prepared 1.5% and 2% spray solutions by placing either 7.5 or 10 ml of product in a 500 ml spray bottle. Based on pilot scale studies, we also added 1 ml (0.25%) of Cide-Kick II non-ionic surfactant to the spray solution. Current label directions suggest the use of 1 to 4 quarts of product in 100 to 400 gallons of water per acre for water hyacinth control. Labels recommend that herbicide applications ensure thorough wetting of the foliage and we sprayed to insure thorough and even coverage of the vegetation growing in each pot. . There were

approximately 5 ml of mixture applied to the surface of the 3.8 L pots. For reference, when applying 200 gallons of spray mixture (2 qts of herbicide and 199.5 gallons of water) per acre, there are ~ 17 ml of mixture applied per square foot.

Following the application of the foliar treatments, one group of pots was submerged in a 900 L tank at 1 day post-treatment. The plants in the other set of pots were not submerged in the tanks until 10 days post-treatment. Untreated reference pots were also submerged at 1 and 10 days. Following placement in the water, plants were given a 4-week recovery period. At this time plants were harvested and all shoot biomass was collected, dried at 70C for 48 hrs, and weighed.

In addition to the emergent foliar treatments, we also submerged a group of 1 L pots containing the emergent form of the variable milfoil and immediately treated with 2,4-D amine and triclopyr at rates of 1.5 mg/L for a 24-hour exposure period. Past studies have demonstrated this rate to be highly effective versus the submersed form of variable milfoil. At the end of the exposure period, plants were removed from the treatment tanks, rinsed thoroughly and moved to a 900 L grow-out tank. These plants were also given a 4-week recovery period. Plants were harvested and analyzed as described above.

Each treatment was replicated four times and data were subjected to analysis of variance. Biomass data were compared to untreated controls via a Dunnett's test ($p< 0.05$). Evaluations were initiated in August 2006 and October 2006 and these data were pooled for analysis.

*Results:*

Treatment of variable milfoil foliage with either 2,4-D or triclopyr resulted in slight epinasty of the small shoots within 1 day after treatment. It is notable that symptoms were not markedly different by 10 days post-treatment. Growth of variable milfoil while in the emergent form is very slow and this likely inhibits the visual activity that is often associated with the auxin-type inhibitors.

Following submersion, untreated control plants began elongating within just a few days. While these plants grew rapidly to the water surface, the variable milfoil treated with 2,4-D and triclopyr showed strong auxin-type symptoms and no evidence of growth during the 4-week recovery period. Both the 1.5 and 2% treatments resulted in removal of viable shoot tissue at the 4-week harvest date (Table 9).

Table 9. Shoot biomass of variable milfoil following treatment with foliar applications of 2,4-D and triclopyr to the emergent form. Following treatment, plants were submerged and given a four-week recovery period. Each biomass value represents four replicate treatments.

| Treatment | Treatment Rate % Solution | # Days Prior to Submersion | Shoot Biomass g/dry wt. /pot |
|---|---|---|---|
| | | | |
| Untreated | 0 | 1 | **4.7 a** |
| Triclopyr | 1.5 | 1 | **0 b** |
| 2,4-D | 1.5 | 1 | **0 b** |
| Triclopyr | 2 | 1 | **0 b** |
| 2,4-D | 2 | 1 | **0 b** |
| | | | |
| Untreated | 0 | 10 | **3.9 a** |
| Triclopyr | 1.5 | 10 | **0 b** |
| 2,4-D | 1.5 | 10 | **0 b** |
| Triclopyr | 2 | 10 | **0 b** |
| 2,4-D | 2 | 10 | **0 b** |
| | | | |

A 24-hour exposure of the emerged form of variable milfoil to an aqueous treatment of 2,4-D and triclopyr at 1.5 mg/L provided some growth regulation, but did not control the plants (Table 10). Auxin-type symptoms were evident during the initial phase of the 4-week recovery period, but the variable milfoil in this treatment increased markedly during this time. Initial shoot biomass of the emerged form was between 500 and 900 mg dry weight per pot. We hypothesize that the cuticle formed to allow survival in the emergent form serves as a short-term barrier to herbicide uptake upon re-flooding. This condition would not be expected to prevail for long, as the plants rapidly revert to a submersed morphology.

Table 10. Shoot biomass of variable milfoil following exposure of the emergent form to aqueous concentrations of 2,4-D and triclopyr. Plants were harvested at 4 weeks post-treatment and each value represents four replicate treatments.

| Treatment | Treatment Conc. mg/L | Exposure Time (hr) | Shoot Biomass, g dry wt./pot |
|---|---|---|---|
| | | | |
| Untreated | 0 | 24 | **4.3 a** |
| Triclopyr | 1.5 | 24 | **3.7 b** |
| 2,4-D | 1.5 | 24 | **2.9 b** |

These results suggest that foliar applications of either 2,4-D or triclopyr to emergent forms of variable milfoil can provide good control once the site is re-inundated with water. The slow growth of the emerged form likely precludes getting complete control of the variable milfoil until water returns to the site.

From an herbicide application perspective, there are both advantages and disadvantages to treating the emergent form of variable milfoil. One key advantage to treating the emergent form is the reduced use of total herbicide per area. For example treatment of 1 acre using an emergent application would result in the use of approximately 2 quarts of herbicides. In contrast, treatment of 1 acre with a water depth of 3 feet at a concentration of 1.5 mg/L of 2,4-D would require the application of 3 gallons of herbicide (or 1 gallon per acre foot of water). Treatment of the emerged form of the variable milfoil would also be preferred in sites where high rates of water flow would result in rapid dilution of herbicide away from the treatment zone, such as in larger rivers and streams. One key disadvantage to treating the emerged form of the plant is the necessity to be sure that all the individual plants are physically treated. Given the small stature of the emerged form, identification of all plants may prove to be one of the bigger challenges.

Based on the data collected to date, treatment of the emerged form of variable milfoil is encouraged to supplement submersed applications. Preventing these plants from re-establishing will help to avert rapid recovery in selected sites.

Figure 14. A 1 Liter pot containing the emerged form of variable milfoil.

*Project Summarization:*

Results of this work suggest that variable milfoil displays a wide range of sensitivity to the various registered and experimental use aquatic herbicides. There were several unexpected findings that included: 1) the enhanced efficacy of the ester vs. amine formulations of 2,4-D; 2) the high level of sensitivity of this plant to the protox inhibitors carfentrazone and flumioxazin; 3) the relative lack of sensitivity of variable milfoil to standard contact herbicides such as diquat, endothall, and copper. As with any plant that can recover from a single node, long-term control with herbicides can prove challenging; however, the choice of herbicide, use rate, and formulation is likely to be a significant factor in determining the efficacy and longevity of control.

**Recommendations:**

Recommendation 1. The NHDES should continue to encourage the use of 2,4-D BEE for control of variable milfoil. This product was the most effective of the auxin-mimic herbicides, and the systemic action should provide the best chance for killing the entire plant.

Recommendation 2. The NHDES should consider evaluating the protox inhibitors carfentrazone and flumioxazin as contact herbicides for variable milfoil control These products were the most effective of the contact herbicides, and the short exposure requirements would allow for the use of these products in small-scale applications.

Recommendation 3. In situations where whole-lake management is warranted, the NHDES may want to further evaluate the slow-acting enzyme inhibitors fluridone and penoxsulam. These products were also effective on the invasive *Cabomba caroliniana*.

Recommendation 4. Early season cool-water applications should be evaluated to determine if this strategy will provide enhanced efficacy or improved selectivity compared to later season applications. The ability to treat low levels of biomass when the plants are in an active growth phase may represent an opportunity to treat the plants at a phenological weak point. This strategy must be balanced against the higher water flow rates that may be characteristic of spring in New England.

Recommendation 5. Late summer or early fall applications should be evaluated with auxin-mimic herbicides to determine if treatment timing can provide enhanced location into the root crown. Our studies suggest that water temperature would be of minimal concern at this time of year.

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APPENDIX 2

# Efficacy of Diquat and Carfentrazone-ethyl on Variable-leaf milfoil

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## INTRODUCTION

Variable-leaf milfoil (*Myriophyllum heterophyllum* Michx.) is a native submersed plant historically ranging from southwestern Quebec and Ontario to North Dakota and southward to New Mexico and Florida (Godfrey and Wooten 1981). This species has recently been introduced to the Northeastern U.S., where it causes many of the same problems associated with Eurasian watermilfoil (*Myriophyllum spicatum* L.) infestations. Variable-leaf milfoil has become particularly problematic in low alkalinity water bodies characteristic of Connecticut, Maine, Massachusetts, and New Hampshire.

Despite ongoing management programs and continued expansion of this invasive species, there is limited information available regarding efficacy of the various registered herbicides for control of variable-leaf milfoil (Getsinger et al. 2003). Therefore a study was conducted to evaluate the efficacy of two contact herbicides registered for aquatic use. Diquat (6,7-dihydrodipyrido{1,2-α:2',1'-*c*]pyrazinediium ion) has been registered for aquatic use since 1961 and it is a rapid acting photosystem I inhibitor that is currently used for operational control of variable-leaf milfoil and numerous other submersed plants. Reports from resource managers indicate that diquat has been somewhat

inconsistent regarding the duration of control of variable-leaf milfoil. While, diquat efficacy can be influenced by factors such as concentration and exposure time, turbidity, stage of plant growth, water temperature, and buildup of epiphytes and inorganic materials on leaf surfaces (Netherland et al. 2000, Hofstra et al. 2001, Poovey and Getsinger 2004,), there is no information on the basic sensitivity of variable-leaf milfoil to this herbicide. Carfentrazone-ethyl (a,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid, ethyl ester) was registered for aquatic use in 2005, and is a rapid-acting protoporphyrinogen oxidase (protox) inhibitor. Carfentrazone is used for broadleaf weed control in terrestrial systems and activity on various submersed species is still under investigation. Recent studies evaluating carfentrazone efficacy on Eurasian watermilfoil and parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.) suggest that this compound is not highly active on these species at rates ranging from 50 to 200 µg ai $L^{-1}$ (Gray et al. 2007, Glomski et al. 2006). The objective of this study was to evaluate the activity of two contact herbicides on variable-leaf milfoil, an emerging invasive plant problem in the Northeastern US.

## MATERIALS AND METHODS

This study was conducted in a greenhouse at the Lewisville Aquatic Ecosystem Research Facility (LAERF) located in Lewisville, TX. Plastic pots (750 mL) were filled with LAERF pond sediment amended with 3 g $L^{-1}$ osmocote (16-8-12). Each pot was planted with two 15 cm tips of variable-leaf milfoil and four pots were placed in each aquarium. Aquariums were filled with a 4:1 ratio of deionized water and alum treated

water from nearby Lake Lewisville. Aquariums were situated in 1000-L fiberglass tanks that were filled with water and served to regulate water temperatures in the experimental aquaria. Water temperatures in the aquariums were maintained at 22 to 24 C by circulating water in the fiberglass tanks through a Pacific Coast Imports C-1000 1 HP chiller. Carbon dioxide was bubbled into each aquarium daily to lower the water pH to 6.5 to better simulate the soft water conditions that are characteristic in the Northeast where variable-leaf milfoil is problematic. Pretreatment biomass was collected and prior to treatment variable-leaf milfoil stems were either at the surface or just below the water surface.

Concentration exposure times for diquat (Reward®, Syngenta Crop Protection, Inc., Greensboro, NC) included 180 and 370 µg ai $L^{-1}$ for 6, 18, and 30 hours. The 370 µg ai $L^{-1}$ rate of diquat represents the maximum use rate of 2 gallons per acre in 4 feet of water. Carfentrazone (Stingray®, FMC Corporation, Philadelphia, PA) treatments were 100 ug ai $L^{-1}$ for 6, 18, and 30 hr and 200 µg ai $L^{-1}$ for 2, 6 and 18 hours. The 200 µg ai $L^{-1}$ rate of carfentrazone represents the maximum use rate of 1.1 gallons per acre in 4 feet of water. At the end of each exposure period, aquaria were flushed with untreated water for 10 minutes to exchange the volume of water in each aquarium three times. Continuous aeration with carbon dioxide was maintained during the treatment period. After that, carbon dioxide was added once a day between 10 am and 2 pm. The pH was monitored throughout the exposure period and ranged from 6.24 to 6.66. The pH was measured once daily following treatment.

At 42 days after treatment (DAT), all viable shoot biomass was harvested from three pots in each tank and plants were dried at 70 C for 48 hr. For statistical analysis,

dry weight values were square root transformed in order to meet the assumptions of normality and equal variance. Transformed data was subjected to analysis of variance (ANOVA). Means were compared using the Student-Newman-Keuls Method (SNK; $P \leq 0.001$). Non-transformed data are presented in the figures comparing post-treatment plant biomass.

## RESULTS AND DISCUSSION

Variable-leaf milfoil grew well during the course of these studies as evidenced by the over 25-fold increase in the pretreatment biomass from $0.19 \pm 0.01$ g to values exceeding 5 g per experimental container (Figures 1 and 2).

*Diquat:* At 15 DAT, plants treated with 370 µg ai $L^{-1}$ diquat for 18 and 30 hours and 180 µg ai $L^{-1}$ for 30 hours exhibited signs of browning. At 30 DAT, diquat treated plants still had green tissue present however, some apical tips had deteriorated. By 42 DAT most diquat treatments were not significantly different than the control and provided unacceptable control of variable-leaf milfoil with percent control ranging from 27 to 65 percent (Figure 1). Diquat treatments of 370 µg ai $L^{-1}$ for 18 and 30 hours had significantly less biomass than the control however, only the 370 µg ai $L^{-1}$ for 30 hour treatment provided good control (85%). Our studies allowed for static exposures in water of very high clarity (NTU < 1), however, a thirty-hour exposure period may be difficult to maintain following many treatment scenarios. Reported dissipation rates in reservoirs vary from 16 to 96 percent 0.5 h after treatment (Yeo 1967). Larger-scale applications of diquat in waters with low turbidity (NTU < 2) did result in maintenance of residues well past the 30 hours tested in this study (Parsons et al. 2007).

Unlike Eurasian watermilfoil, which is highly susceptible to diquat, variable-leaf milfoil was only moderately susceptible to diquat at the maximum label rate following extended exposure periods. Skogerboe et al. (2006) reported 97 to 99 percent control of Eurasian watermilfoil at 185 and 370 µg ai $L^{-1}$ with half-lives of just 2.5 and 4.5 h. Therefore, diquat treatments that would provide near complete control of Eurasian watermilfoil would have limited impact on variable-leaf milfoil. Even though Eurasian watermilfoil and variable-leaf milfoil are in the same plant family (Halogoraceae) and genus (*Myriophyllum*) they responded quite differently to diquat. This differing response to diquat among plants in the same plant family has also been reported for members of the Hydrocharitaceae (Glomski et al. 2005).

*Carfentrazone:* Within four days of treatment, all carfentrazone treated plants exhibited bleached or brown apical tips. By 15 DAT, most carfentrazone treated plants were starting to deteriorate however all treatments had shoot regrowth from the root crown present at the time of harvest. Despite this regrowth, all carfentrazone treated plants had significantly less biomass than the untreated control at the harvest period (Figure 2). Regrowth from plant tissue not initially killed is a common response when treating with contact herbicides due to limited translocation throughout plant tissues (Lembi and Ross 1985). Rates of 100 µg ai $L^{-1}$ for 6 to 30 hours provided 61 to 81 percent control and 200 µg ai $L^{-1}$ for 2 to 18 hours provided 64 to 79 percent control. There were no significant differences between concentration-exposure times. Doubling the rate from 100 to 200 µg ai $L^{-1}$ and extending exposures did not improve efficacy. This lack of a rate response to carfentrazone was also seen in Eurasian watermilfoil under static conditions (Glomski et al. 2006). These data suggest that carfentrazone is a

very rapid acting herbicide and that traditional concentration and exposure relationships may not best explain the activity of this protox inhibitor on variable-leaf milfoil. The lack of both a concentration and exposure effect for carfentrazone suggests that lower rates and shorter exposures could be efficacious and need to be tested. The results of this study would suggest that carfentrazone should be evaluated in the field for control of variable-leaf milfoil.

In conclusion, only diquat at 370 µg ai $L^{-1}$ for 30 hours provided good control (85%) of variable-leaf milfoil. Due to the potential for rapid binding to particulates or dissipation of diquat in the field, a 30 hour exposure may not be possible. The strong difference in response between variable-leaf and Eurasian watermilfoil to diquat suggests that variable-leaf milfoil has a higher tolerance to diquat. The combination of a plant that is not highly sensitive and a molecule that would require an extended exposure in order to provide control, may ultimately limit the use of diquat for variable-leaf milfoil control. All rates and exposures of carfentrazone significantly reduced variable-leaf milfoil biomass, however, shoot regrowth from root crowns will require follow-up applications. In contrast to diquat, carfentrazone is much weaker against Eurasian watermilfoil when compared to variable-leaf milfoil.

## ACKNOWLEDGMENTS

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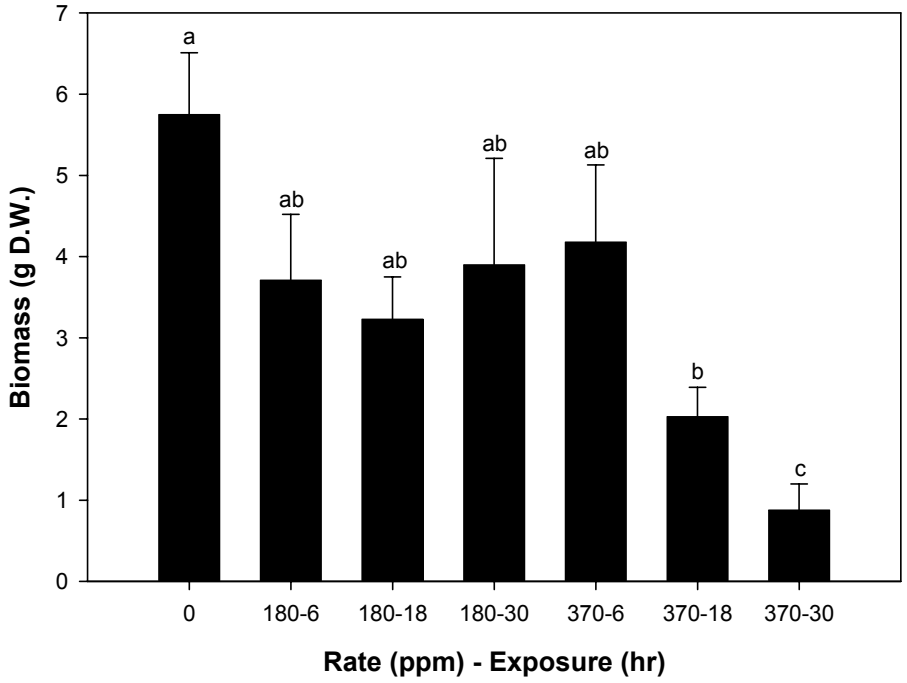
**Footnotes**

[1] SpecPro Inc., U.S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility, 201 E. Jones St., Lewisville, TX 75057; leeann@laerf.org

[2]U.S. Army Engineer Research and Development Center, 3909 Halls Ferry Rd., Vicksburg, MS 39180. Received for publication Dec 20, 2006 and in revised form May 19, 2007.

**List of Figures**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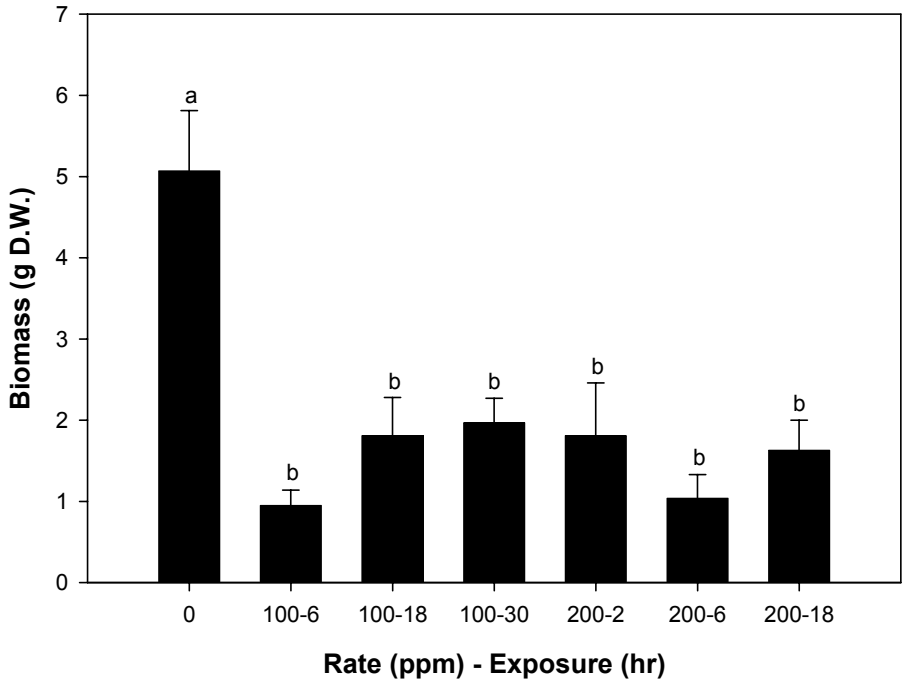
4
3
2
1
0
a
ab
ab
ab
ab
b
c
0
180-6
180-18
180-30
370-6
370-18
370-30
Rate (ppm) - Exposure (hr)


Biomass (g D.W.)
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b
b
b
b
b
b
0
100-6
100-18
100-30
200-2
200-6
200-18
Rate (ppm) - Exposure (hr)

APPENDIX 3

**Efficacy of Fluridone and Three ALS Inhibitors on Variable-leaf Milfoil**

LeeAnn M. Glomski[1] and Michael D. Netherland[2]

## ABSTRACT

Variable-leaf milfoil (*Myriophyllum heterophyllum* Michx.) is native to the United States, but is considered an invasive species in the northeastern United States. It can cause some of the same problems as the non-native Eurasian watermilfoil (*Myriophyllum spicatum* L.) such as shading out other native submersed plants and impeding recreational activities. A study was conducted to determine the efficacy of fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone), bispyribac-sodium (2,6-bis[(4,6-dimethoxy-2-pyrimidinyl)oxy]benzoic acid), imazamox (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid, ammonium salt), and penoxsulam (2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl) benzenesulfonamide) on variable-leaf milfoil. Of the four herbicides tested, fluridone and penoxsulam were the most effective. Fluridone at 5 µg ai $L^{-1}$ caused a significant decrease in variable-leaf biomass; however, increasing the rate 2, 4 or 10 times did not cause greater decreases in biomass. Penoxsulam at 5 µg ai $L^{-1}$ also caused a significant decrease in biomass and increasing the rate 2 and 4 times did cause greater reductions in biomass. Increasing the rate of penoxsulam from 20 to 50 µg ai $L^{-1}$ did not improve efficacy.

**Key Words:** *Myriophyllum heterophyllum,* penoxsulam, imazamox, bispyribac, aquatic herbicides

## INTRODUCTION

Variable-leaf milfoil is a native perennial submersed plant ranging from southwestern Quebec and Ontario to North Dakota and southward to New Mexico and Florida (Godfrey and Wooten 1981). It is classified as a species of concern in Kentucky and is endangered in Ohio and Pennsylvania (USDA 2007). In the northeastern U.S. however, variable-leaf milfoil is not native and is considered an invasive and weedy species. Variable-leaf milfoil is listed as invasive in states such as Connecticut and Maine, prohibited in Massachusetts, and is a class A noxious weed in Vermont (USDA 2007). As an invasive species, it causes many of the same problems as Eurasian watermilfoil (*Myriophyllum spicatum* L.), including shading out other native submersed vegetation and interfering with recreational activities and water supplies (Halstead et al. 2003, NH-DES 2002). It has also been estimated that variable-leaf milfoil could reduce lake-front property values by as much as 20 to 40 percent in New Hampshire (Halstead et al. 2003). Variable-leaf milfoil is an aggressive invader that can grow up to one inch per day under optimal nutrient, temperature and light conditions and spreads mainly via fragmentation (NH-DES 2002).

To date, there has been some indication that auxin-type herbicides are effective at controlling variable-leaf milfoil. Research by Getsinger et al. (2003) found that triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) was effective at controlling variable-leaf milfoil at a wide range of rates and exposure times. Bugbee et al. (2003) reported good

control of variable-leaf milfoil treated with 2,4-D ester [(2,4-dichlorophenoxy)acetic acid] in the field. Although effective, both triclopyr and 2,4-D have use restrictions for drinking water and therefore may not be a viable option for treating around portable water intakes. Possible alternatives for controlling variable-leaf milfoil in situations where triclopyr and 2,4-D cannot be used include fluridone, bispyribac, imazamox and penoxsulam.

Fluridone has been registered by the U.S. Environmental Protection Agency (USEPA) under a Section 3 nationwide use for more than 30 years. Bispyribac, imazamox, and penoxsulam currently have Experimental Use Permits (EUPs) for aquatic use issued by the USEPA. Because these products impact plant-specific enzymes, they do not have major impacts on non-target insects or animals. As such, they have favorable toxicology profiles that will likely preclude any restrictions on using the water for drinking, fishing, or swimming.

Fluridone inhibits the enzyme phytoene desaturase (PDS) in the carotenoid biosynthetic pathway. Carotenoids play an important role in preventing photooxidative damage by quenching chlorophyll triplets that would lead to oxygen singlets (Bartley and Scolnik 1995). Without carotenoids, new plant tissue becomes bleached due to photodestruction of chlorophyll (Bartels and Watson 1978). Bispyribac, imazamox and penoxsulam inhibit the enzyme acetolactate synthase (ALS), which is involved in biosynthesis of the branched-chain amino acids leucine, isoleucine, and valine. Without these enzymes, protein synthesis and growth are inhibited, ultimately causing plant death (WSSA 2002). The impact of these slow acting enzyme inhibitors on plants such as hydrilla (*Hydrilla verticillata* (L.f.) Royle) and Eurasian watermilfoil is most notable on

the actively growing shoot meristems; extended exposure (>45 days) to phytotoxic concentrations of PDS and ALS inhibitors is required to achieve plant control (Netherland et al. 1993, Nelson et al. 1993, Langeland 1993). The objective of this study was to determine the efficacy of fluridone, bispyribac, imazamox and penoxsulam on variable-leaf milfoil.

## MATERIALS AND METHODS

Study 1

This study was conducted in the greenhouse at the US Army Engineer Research and Development Center Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. Plastic pots (750 mL) were filled with LAERF pond sediment amended with 3 g $L^{-1}$ Osmocote (16-8-12). Each pot was planted with either two 15-cm apical tips of variable-leaf milfoil. Pots were topped with a 1-cm layer of play sand and two pots of each species were placed in each aquarium. Aquaria were filled with alum-treated Lake Lewisville water. Eight aquaria were situated into each of eight 1000-L fiberglass tanks filled with water. Water temperatures were maintained at 22 to 24 C. Carbon dioxide was bubbled into each aquarium once a day to drop the pH to 6.5.

Ten days after planting, aquaria were treated with one of the following herbicides: bispyribac, fluridone, imazamox, or penoxsulam. Rates of bispyribac, fluridone, and penoxsulam were 5, 10, and 20 µg ai $L^{-1}$; imazamox rates were 10, 25, and 50 µg ai $L^{-1}$. Treatments were randomly assigned and replicated 4 times. Treatment rates evaluated were chosen based on current proposed use rates for the ALS inhibitors and current

recommended use rates for fluridone. After treatment, herbicides were left in the aquaria for a static exposure.

At 42 days after treatment (DAT) all viable shoot biomass was harvested, dried at 65 C, and weighed. Dry weight values for bispyribac, fluridone, and penoxsulam were transformed by squaring the data in order to meet the assumptions of normality and equal variance. Imazamox data was analyzed separately because of the different rates were tested. Both data sets were subjected to one-way analysis of variance (ANOVA). Means were compared using the Student-Newman-Keuls Method (SNK; $P \leq 0.001$).

Study 2

This study was set-up in the same manner as study 1; however, four pots of variable-leaf milfoil were planted in each aquarium. Ten days after planting, aquaria were treated with one of the following herbicides: bispyribac, fluridone, imazamox, or penoxsulam. Rates of fluridone and penoxsulam were 2.5, 5, 10, 20 and 50 µg ai $L^{-1}$; bispyribac rates were 20 and 50 µg ai $L^{-1}$ and imazamox rates were 25 and 75 µg ai $L^{-1}$. Treatments were randomly assigned and replicated 4 times.

At 50 days after treatment (DAT) all viable shoot biomass was harvested, dried at 65 C and weighed. Bispyribac and imazamox data were transformed by squaring the data in order to meet the assumptions of normality and equal variance. The data was subjected to analysis of variance (ANOVA). Means were compared using the Student-Newman-Keuls Method (SNK; $P=0.169$). Fluridone and penoxsulam data were analyzed individually and subjected to regression analysis.

## RESULTS AND DISCUSSION

Both fluridone and penoxsulam were active on variable-leaf milfoil, while bispyribac and imazamox treatments showed limited activity at rates tested (Figures 1-3). Plants treated at 50 and 75 µg ai $L^{-1}$ imazamox and at 50 µg ai $L^{-1}$ bispyribac did not reach the water surface, with both showing increased production of lateral meristems and slight curling of apical tips at the time of harvest. While both compounds resulted in reduced plant height, neither showed strong potential in controlling variable-leaf milfoil, and effects would be described as growth regulating.

Penoxsulam and fluridone were much more active against variable-leaf milfoil (Figures 1, 4, and 5). The lowest rate of each herbicide (2.5 µg ai $L^{-1}$) reduced biomass by about 27 percent. Fluridone at 5 µg ai $L^{-1}$ reduced variable-leaf milfoil biomass by 75 and 87 percent in both studies; rates higher than 5 µg ai $L^{-1}$ did not improve control. However, fluridone symptoms appeared sooner in plants treated at higher rates but by the time of harvest, all treatments looked similar visually. This lack of an improved response to increasing use rates of fluridone has also been described for Eurasian watermilfoil and hydrilla (Netherland and Getsinger 1995).

Penoxsulam controlled variable-leaf milfoil by 27 to 91 percent in both studies and control increased as rate increased up to 20 µg ai $L^{-1}$, after which control leveled off. Variable-leaf milfoil treated at 2.5 µg ai $L^{-1}$ had grown to the water surface and at 5 µg ai $L^{-1}$ plants were vibrant and were just six inches below the water surface at the time of harvest. Plants treated at 10 and 20 µg ai $L^{-1}$ had collapsed in the water column one week prior to harvest and started to decompose. Plants treated at 50 µg ai $L^{-1}$ penoxsulam had little to no new growth after treatment and were starting to decompose at the time of harvest.

Of the four herbicides tested only fluridone and penoxsulam showed potential for controlling variable-leaf milfoil at concentrations currently labeled or proposed for EUP aquatic labels. While these laboratory results suggest that variable-leaf milfoil is quite sensitive to low use rates of fluridone, reports from aquatic plant managers suggest that fluridone has provided inconsistent operational control of variable-leaf milfoil in the field. Prior fluridone studies in outdoor mesocosms demonstrated that treatment timing is crucial for some fluridone-sensitive species such as elodea (*Elodea canadensis* Michx.), while treatment timing had much less impact on Eurasian watermilfoil (Netherland et al. 1997). Our laboratory studies suggest that plants like variable-leaf milfoil, elodea, Brazilian elodea (*Egeria densa* Planch.), and cabomba quickly grow to the water surface, where growth rates slow dramatically and do not form dense entangled canopies similar to Eurasian watermilfoil and hydrilla. Treatment with fluridone when growth rates have decreased would result in reduced symptoms and less stress on the plant; the current laboratory data would suggest that early treatment of actively growing variable-leaf milfoil with fluridone might override the importance of treatment rate. This treatment timing phenomenon needs to be investigated for the ALS inhibitors.

## ACKNOWLEDGMENTS

The authors would like to thank Kristin Dunbar for her assistance during this study and Angela Poovey and Gary Dick for early reviews of this article. Permission was granted by the Chief of Engineers to publish this information. Citation of trade names

does not constitute an official endorsement or approval of the use of such commercial products.

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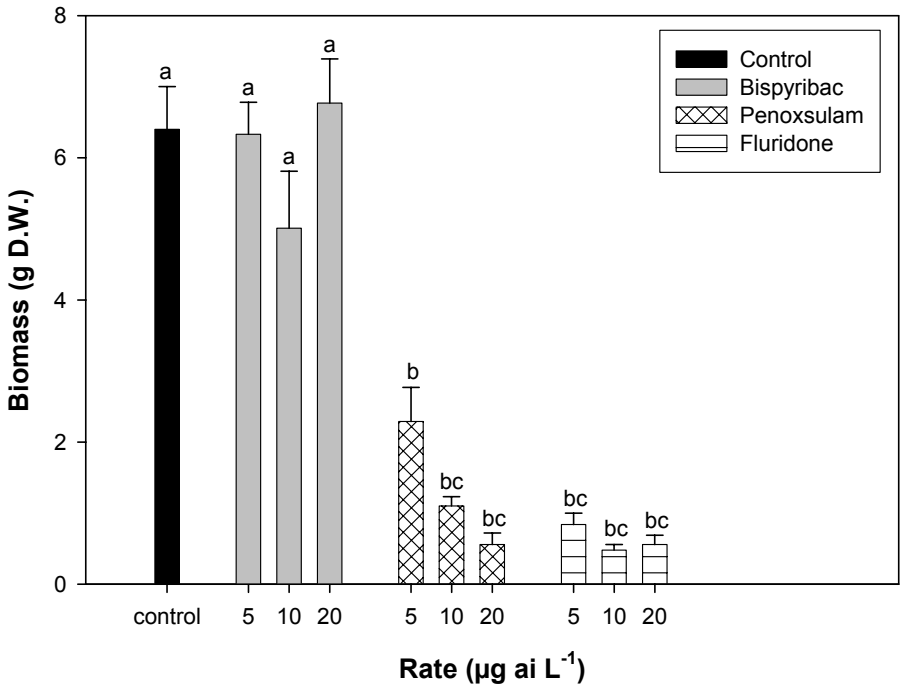
**Footnotes**

[1]U.S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility, 201 E. Jones St., Lewisville, TX 75057; leeann@laerf.org

[2]U.S. Army Engineer Research and Development Center, 3909 Halls Ferry Rd., Vicksburg, MS 39180. Received for publication _____ and in revised form _____.

**List of Figures**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Penoxsulam
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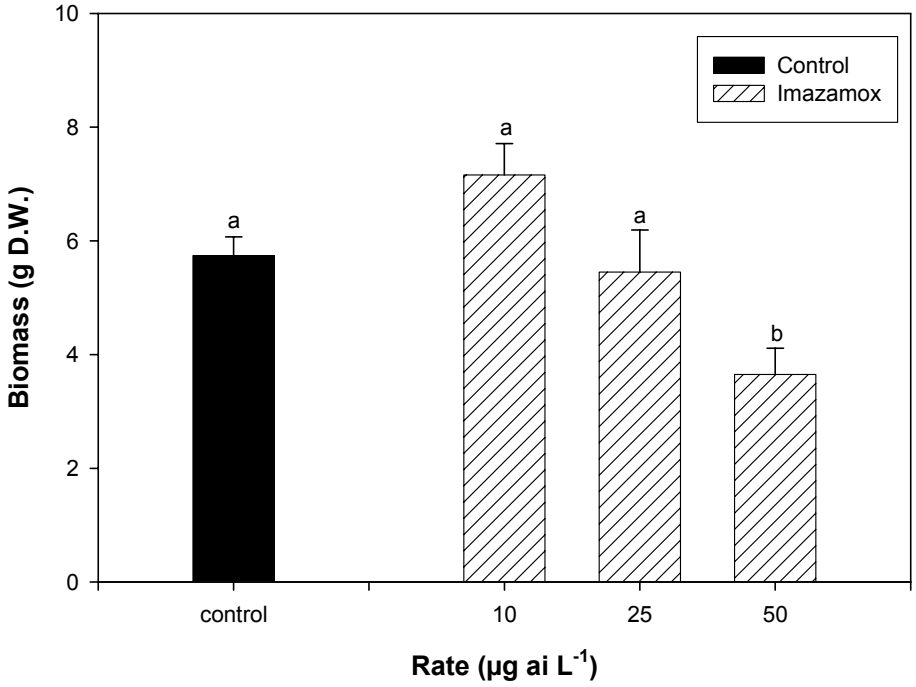
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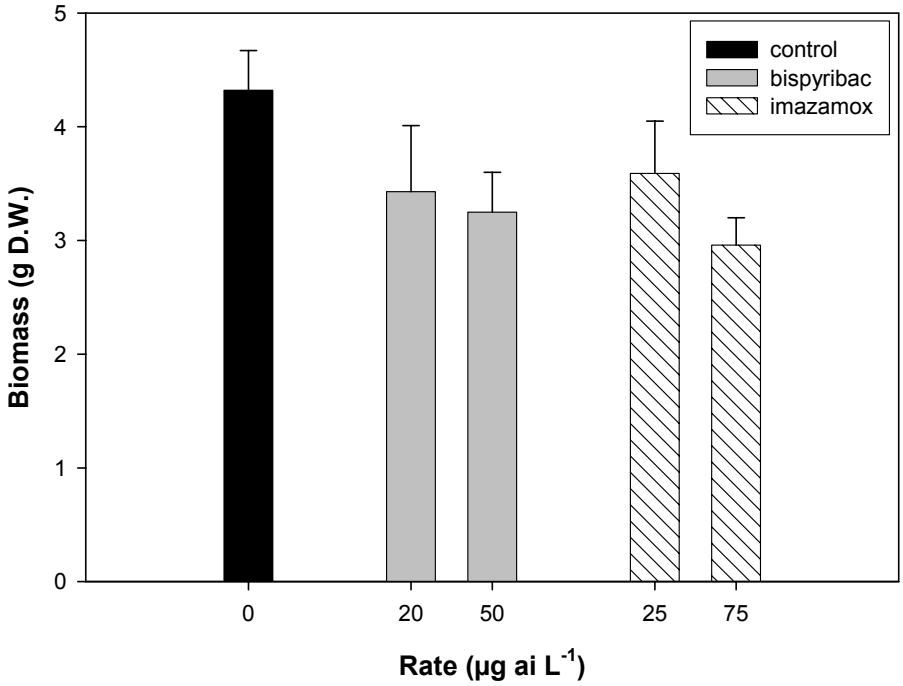
control
bispyribac
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2
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4
5
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20
50
25
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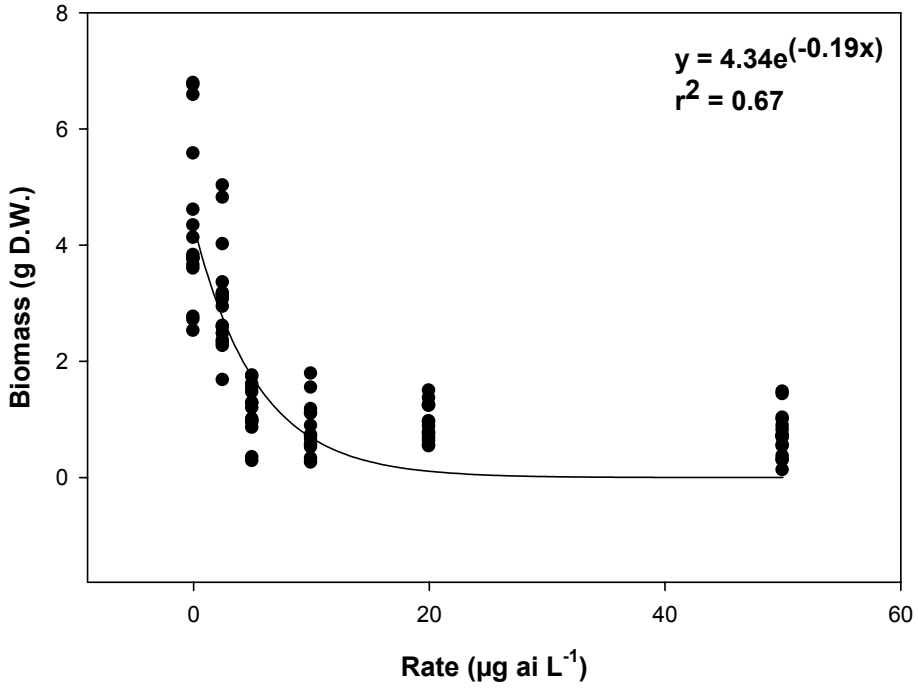
Rate (µg ai L-1)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0
2
4
6
8
0
20
40
60

APPENDIX 4

# Effect of Temperature on 2,4-D Ester and Carfentrazone-ethyl Applications for Control of Variable-leaf Milfoil

LeeAnn M. Glomski[1] and Michael D. Netherland[2]

## INTRODUCTION

Variable-leaf milfoil (*Myriophyllum heterophyllum* Michx.) is a native perennial submersed plant from southwestern Quebec and Ontario to North Dakota and southward to New Mexico and Florida (Godfrey and Wooten 1981). In the Northeastern U.S., variable-leaf milfoil is not native and is considered an invasive and weedy species. As an invasive species, it causes many of the same problems that Eurasian watermilfoil (*Myriophyllum spicatum* L.) does. These problems include shading out native submersed vegetation and interfering with recreational activities and water supplies (Halstead et al. 2003, NH-DES 2002). Variable-leaf is an aggressive invader that can grow up to one inch per day under optimal nutrient, temperature and light conditions and spreads mainly via fragmentation (NH-DES 2002).

Two herbicides that have been shown to effectively control variable-leaf milfoil include 2,4-D ester [(2,4-dichlorophenoxy)acetic acid] and carfentrazone-ethyl (a,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid, ethyl ester). 2,4-D is an auxin-type herbicide effective on dicotyledons (dicots). Although the mode of action is not completely understood, it is believed to involve nucleic acid metabolism and cell wall plasticity. It is thought to

stimulate membrane-bound ATPase proton pumps which acidify the cell wall. Lowering the apoplasmic pH increases the activity of enzymes involved in cell wall loosening causing cells to elongate. 2,4-D also stimulates ethylene production which can cause epinastic symptoms typical of auxin-type herbicides. 2,4-D is rapidly translocated in plant tissues and accumulates in the meristematic regions. Degradation of 2,4-D is via microbial degradation. Carfentrazone is a fast-acting protox inhibitor. It inhibits the enzyme protoporphyrinogen oxidase in chlorophyll synthesis. Inhibition of this enzyme leads to the buildup of phytotoxic intermediates which causes cell membrane disruption. Carfentrazone is a contact herbicide used to control dicots in terrestrial systems and activity on various submersed species is still under investigation. Carfentrazone degrades via hydrolysis (WSSA 2002).

In greenhouse studies, 2,4-D ester at rates of 500 and 1500 µg ai $L^{-1}$ with exposure periods of 3, 8, and 24 hours provided 98 to 100 percent control of variable-leaf milfoil (Glomski and Netherland unpublished data). Bugbee et al. (2003) also reported that 227 kg $ha^{-1}$ 2,4-D ester as Navigate© controlled nearly all the variable-leaf milfoil in treated field sites. Variable-leaf milfoil exposed to 100 µg ai $L^{-1}$ carfentrazone for 6 to 30 hours was reported to provide 61 to 81 percent control and doubling the rate did not improve efficacy (Glomski and Netherland in review). There is currently no information in the literature regarding field applications of carfentrazone to control variable-leaf milfoil. The objective of this study was to determine the effect temperature has on carfentrazone-ethyl and 2,4-D ester applications for control of variable-leaf milfoil.

## MATERIALS AND METHOD

This study was conducted in the greenhouse at the US Army Engineer Research and Development Center Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. Plastic pots (750 mL) were filled with LAERF pond sediment amended with 3 g $L^{-1}$ osmocote (16-8-12). Each pot was planted with two 15 cm apical tips of variable-leaf milfoil. Pots were topped with a 1 cm layer of play sand and four pots were placed in each aquarium. Aquariums were filled with alum treated Lake Lewisville water. Aquariums were situated in 1000-L fiberglass tanks filled with water and water temperatures in the aquariums were maintained at 18 to 20 C by circulating water in the fiberglass tanks through a Pacific Coast Imports C-1000 1 HP chiller. Carbon dioxide was bubbled into each aquarium once a day to lower the pH to 6.5 simulating soft water conditions that are characteristic in the Northeast where variable-leaf milfoil is problematic.

Forty-one days after planting, water temperatures were slowly adjusted to 13, 16, 19, and 22 C in the aquariums. Once temperatures stabilized, tanks were treated at 100 µg ai $L^{-1}$ carfentrazone (Stingray, FMC Corporation, Philadelphia, PA), 250 µg ai $L^{-1}$ 2,4-D ester (Aquakleen, Cerexagri, Philadelphia, PA), or 500 µg ai $L^{-1}$ 2,4-D ester. Treatments were replicated four times and included an untreated control. Carfentrazone treatments were static exposures whereas 2,4-D ester applications were for a 3 hr exposure. Two DAT, temperatures were brought back up to 21 C to stimulate growth following the exposure.

At 28 days after treatment (DAT) all viable shoot biomass was harvested and dried at 65 C. Data was subjected to a two-way analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

Two DAT all treated plants at 19 and 22 C were showing symptoms. Carfentrazone treated plants had bleached tips and dark red to brown stems, and stems of plants treated with 2,4-D were curling. No symptoms were present in tanks at 16 and 13 C. At 10 DAT, carfentrazone treated plants at all temperatures were necrotic and starting to collapse. Symptoms of 2,4-D exposure were also now present on plants exposed to 16 C. By 21 DAT, all treated plants at 22 C were dead. At 19, 16 and 13 C only the 250 ppb 2,4-D and the carfentrazone treated plants still had a small amount of viable tissue present.

There was no interaction between herbicide treatment and temperature and there were no differences among the temperatures tested. All treatments were significantly different than the untreated control (Fig. 1). There were no differences between carfentrazone and 2,4-D or between 250 and 500 ppb 2,4-D. All three herbicide treatments reduced variable-leaf biomass by 96 to 100 percent.

Lack of a temperature effect on 2,4-D applications has also been seen in the field. Bugbee et. al (2003) reported good control of variable-leaf milfoil regardless of month of application (May, June, July and September). Results from this study indicate that temperature was not a significant factor in carfentrazone or 2,4-D efficacy when used for variable-leaf milfoil control. Results suggest that applications could take place in early spring when water temperatures are cooler. Treating the variable-leaf milfoil before it tops out and before native species begin actively growing are two advantages to early spring applications. The results of this study would suggest that carfentrazone should be evaluated in the field for control of variable-leaf milfoil.

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**LITERATURE CITED**

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**Footnotes**

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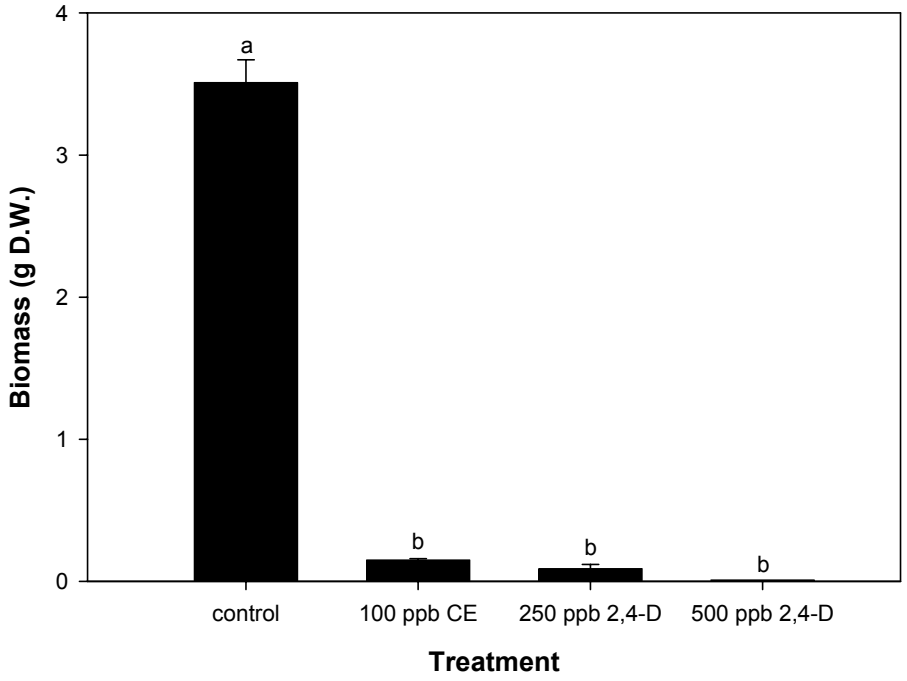
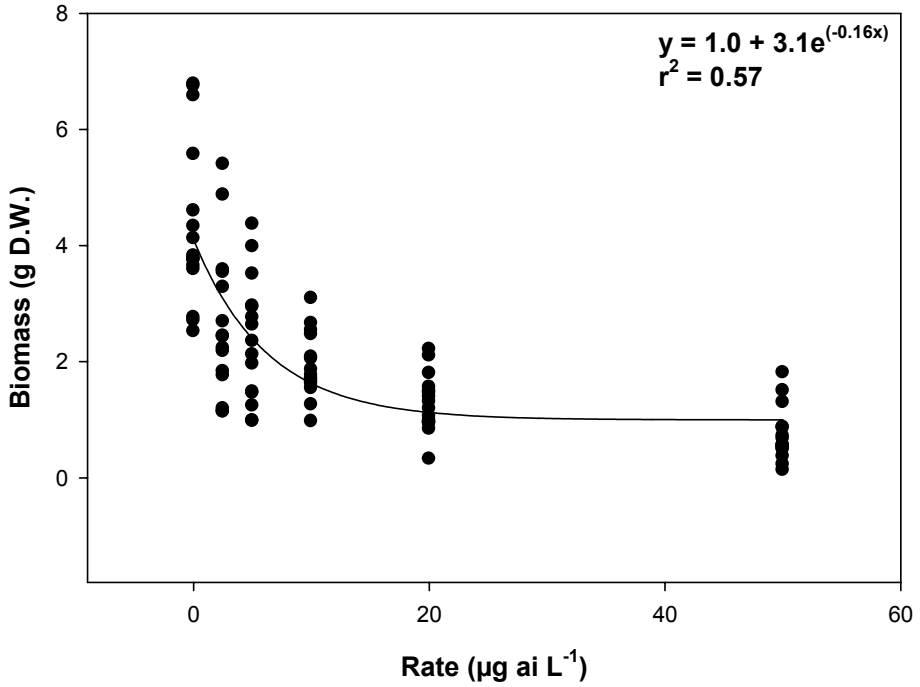
**Figure Legend**

Figure 1: Mean (± SE) dry weight of variable-leaf milfoil biomass 28 days after treatment with carfentrazone (CE) and 2,4-D ester. There was no interaction between herbicide treatment and temperature and no differences among the temperatures. Bars sharing the same letter do not significantly differ from each other.


4
3
2
1
0
Biomass (g D.W.)
a
b
b
b
control
100 ppb CE
250 ppb 2,4-D
500 ppb 2,4-D
Treatment


y = 1.0 + 3.1e(-0.16x)
r2 = 0.57
Biomass (g D.W.)
Rate (µg ai L-1)
0
2
4
6
8
0
20
40
60

APPENDIX 1. A list of registered and Experimental Use aquatic herbicides evaluated against variable milfoil.

| Compound | Date Registered | Submersed Use for aquatics | Comments | Chemical Name |
|---|---|---|---|---|
| Copper<br>Copper chelates | 1950's | Yes | Major use for algae control, but Also used in combination with aquatic herbicides | copper ethylenediame complex |
| 2,4-D | 1959 (ester)<br>1976 (amine) | Yes | Systemic for submersed dicots such as Eurasian watermilfoil | (2,4-dichlorophenoxy) acetic acid |
| Endothall | 1960 | Yes | Contact herbicide. Alternative to fluridone for resistant hydrilla | 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid |
| Diquat | 1962 | Yes | Contact herbicide. | 6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium ion |
| Fluridone | 1986 | Yes | Large-scale or whole-lake management | (1-methyl-3-phenyl-5-[-3(trifluoromethyl)phenyl]-4(1H)-pyridinone |
| Triclopyr | 2002 | Yes | Systemic for submersed dicots | [(3,5,6-trichloro-2pyridinyl)oxy]acetic acid |
| Carfentrazone- | 2004 | Yes | Contact herbicide. Dicots | ethyl α, 2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate |
| Penoxsulam | 2007 | Yes | Just Registered: Hydrilla, Floating Plants , Use pattern Similar to fluridone | (2-(2,2-difluoroehtoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5c] pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide) |

## EXPERIMENTAL USE PERMITS

| | | | | |
|---|---|---|---|---|
| *Imazamox* | *2005* | *Yes* | *Under Experimental Use* Permit. Section 24c Label for hydrilla in FL. Submersed use similar to fluridone and emergent use similar to imazapyr | (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-Imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid) |
| *Bispyribac* | *2006* | *Yes* | *Under Experimental Use Permit Use pattern similar to fluridone.* | 2,6-bis[(4,6-dimethoxy-pyrimidin-2-yl)oxy]benzoate) |
| *Flumioxazin* | *2006* | *Yes* | *Under Experimental Use* Permit. Rapid contact activity | (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propymyl)-2H-1,4-b benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)- |
| *Quinclorac* | *2007* | *Yes* | *Under Experiment Use Permit* Auxin-mimic | 3,7-dichloro-8-quinolinecarboxylic acid |